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Degree: Doctor of Philosophy

Year this Degree Granted: 2000

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Water flow and growth of aspen (Populus tremuloides) at low soil temperatures* submitted by *Xianchong Wan* in partial fulfillment of the requirements for the degree of *Doctor of Philosophy* in *Forest Biology and Management*.

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ABSTRACT

Low soil temperature inhibited aspen (*Populus tremuloides*) root growth and lowered root water flow. The reduced water flow at low temperatures appeared to trigger a decrease in stomatal conductance, net assimilation and shoot water potential. The root water flow was positively correlated with the leaf size, total leaf area and shoot length. The reduction of root water flow at low soil temperatures was a result of a decrease in root hydraulic conductivity (L_p) and root surface area. Resistance to water flow through the roots increased with decreasing root temperature. This increase could not be fully explained by an increase in water viscosity at low temperature. The shapes of Arrhenius plots of root water flow and the activation energies (E_a) were dependent on the direction, the sequence and the extent of temperature change. These plots suggested that one or more chilling-sensitive processes in root water transport were not quickly reversible. The responses of roots to low temperature were not a consequence of cell membrane injury since the reduction of root temperature to as low as 4°C did not affect root membrane electrolyte permeability.

I employed mercurial reagents to study water channel proteins in aspen roots. The results demonstrated that mercury-sensitive processes in aspen roots play a significant role in regulating plant water balance by their effects on root hydraulic conductivity. Water transport via the water channels was more temperature-sensitive than that through lipid bilayers.

Exogenous ABA applied to the roots and excised shoots of aspen inhibited stomatal conductance proportionately to the applied concentration. Compared with the intact seedlings, stomatal conductance in the excised shoots was more sensitive to ABA. More than 10% of the ABA concentration applied to the roots was found in the xylem exudates of root systems exposed to 0.3 MPa hydrostatic pressure. A similar concentration of ABA applied to the excised shoots produced a faster and deeper reduction of stomatal conductance. ABA applied to the roots had no effect on root steady-state flow rate over the five-hour experimental period. Similarly, ABA had no effect on root hydraulic conductivity and the activation energy of root water flow rates.

DEDICATION

To my parents, Zhou-Qing Wan and Bao-Xiu Xiong, for their love and support.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Janusz Zwiazek, for his giving me the opportunity to pursue my further studies here. I will remember all his encouragement and support. I owe a debt of gratitude to Prof. Chao Qisheng, the former President of Nanjing Forestry University, for his supporting me to leave for the studies. I would also like to acknowledge Dr. Victor Lieffers, a member of my supervisory committee, for his carrying most of my personal financial support and initiating my thesis project.

I am grateful to Sylvie Renault, Simon Landhaüsser, Yinfang Wang and Ken Stadt for their help, especially during my early days in this Department. I would like to thank Darlene Saunders for her warm-hearted help. I appreciate my lab fellows for their friendship and help.

A lot of thanks go to my Chinese Canadian friends, Weixing Tan, Runpeng Wei, Jianyong Jiang, for their encouragement throughout my graduate studies. They provide much relief from the stresses of graduate student life and the new continent life.

This research was financially supported by NSERC (National Science and Engineering Research Council) (to Dr. Zwiazek) and NCE- SFM (National Centre of Excellence-Sustainable Forest Management) (to Dr. Lieffers).

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CHAPTER ONE

Introduction and Literature Review

INTRODUCTION

Water is the single most important environmental factor affecting plant growth, development and distribution (Kramer 1983). Water is important to plants because of the crucial role that it plays in all physiological processes and because of the large quantities that are required to fulfill these functions. Water balance in plants depends on the rates of water absorption and water loss. Most of the water absorption takes place through roots while most of the water loss from plants is via leaf transpiration. The principal environmental factors affecting the absorption of water are the availability of soil water, soil temperature, and soil aeration (Kramer 1983). In high altitudes and high latitudes, cold soil is an important ecological factor (Tryon and Chapin 1983, Bonan 1992). Of all the water absorbed by plants, about 95% is lost by transpiration and 5% or less is used for metabolic and growth processes (Kramer 1983). Stomata are important in controlling water loss because most of the water that enters plants escapes through the stomata. Stomatal conductance depends on numerous factors including the availability of moisture in the soil and on vapor pressure in the air (Kramer 1988, Davies et al 1986, Passioura 1988), as well as on the water potential difference between the soil and the air. Increasing transpiration causes increased water absorption and decreasing transpiration causes decreased absorption. Conversely, if absorption is reduced by factors such as cold or drying soil, the root water potential is reduced and this reduction is transmitted to the leaves in the xylem sap, where decreasing water potential causes stomatal closure and reduction in transpiration (Kramer 1983). However, stomatal conductance may also respond to root water status independently of the shoot water potential (Termaat et al 1985, Davies et al 1986, Zhang et al 1987).

Trembling aspen (*Populus tremuloides* Michaux) is the most widely distributed native tree species in North America and it is a major component of the boreal forest (Burn and Honkala 1990). Because of the development of pulping technology (Johal and Hatton 1992), aspen has become an economically important species. However, low soil temperature impacts aspen suckering and growth of suckers in boreal environments (Zasada and Schier 1973). This species exhibits reduced growth at low soil temperatures under well-fertilised conditions (Landhäusser and Lieffers 1998). Numerous cases of early aspen dieback in mature stands are spotted in the boreal forest. The causes of dieback are not clear. Since tree water relations are affected by cold soils, it is possible that the reduced growth in cold soils could be due to the inability of roots to supply adequate amounts of water for the tree. It is also conceivable that one of the reasons for dieback could be water imbalance during the periods of unusually high spring air temperatures when soils are still cold. In the present study, I examined water relations and growth of aspen seedlings exposed to low root temperatures.

Objectives and hypotheses

The objectives of the present study were:

1) To investigate the effects of low soil temperature on aspen growth and water relations and to determine a relationship between water uptake and shoot growth.
2) To investigate the mechanisms of water flow inhibition at low soil temperature during the growth season.
3) To test for the existence of water channels in aspen roots and determine their role in regulating water transport through aspen roots.
4) To determine the effects of ABA on root water flow.

I tested the following hypotheses:

1) Reduced water uptake inhibits shoot growth of aspen subjected to low soil temperature.
2) Water channels exist in root cell membranes and regulate water flow.
3) ABA regulates water balance by affecting root hydraulic conductivity.

LITERATURE REVIEW

1. The distribution and biology of aspen

Aspen is a widely distributed species, its range extending from west to east coast in Canada, and from Alaska to Colorado (Figure 1-1). Aspen reproduces by both sexual and asexual methods. The flowers of aspen are typically imperfect (unisexual) and the trees dioecious (Peterson and Peterson 1992). The trees produce a good seed crop very year, however, microenvironment requirements for seedling establishment are stringent, which restricts sexual reproduction (Maini 1968). Vegetative reproduction is the primary means for aspen regeneration (Schier 1981). Most aspen regeneration is by root suckers (Heeney et al 1980). Shoots which arise from adventitious buds on the roots of the parent tree remain connected by parent roots, even after they have developed their own root systems (Cook 1983). Most suckers are formed during the first growing season after a major disturbance such as fire or harvesting (Heeney et al 1980). The prolific suckering produces many ramets (individual shoots) whose root systems remain connected for many years (Shepperd 1993). These ramets, with their root connections, form an individual clone. Aspen root systems are widely spread, with most roots concentrated in a zone between 5 and 20 cm below ground surface (Strong and La Roi 1983). This species achieves its best growth on moist but well-drained soil (Haeussler and Coates 1986), although it can adapt to a wide range of sites (Corns 1989)

2. Low temperature responses in forest species

Chilling injury has been defined as injury at temperatures low enough to cause damage without freezing of water (Levitt 1980). Lyons and Raison (1970) proposed a hypothesis that attempted to unify the diverse observations on chilling injury and acclimation of plants to chilling temperatures. They suggested the primary event in chilling injury is an alteration in the state of a cellular membrane from a relatively fluid liquid-crystalline state to a less fluid gel state. The change from liquid-crystalline to gel phase decreases membrane permeability and changes the activity of membrane-associated enzymes and enzyme systems which results in major alterations of metabolism. A major source of supporting evidence for the above hypothesis came from the analysis of a variety of physiological and biochemical processes with Arrhenius plots (McMurdo and Wilson 1980). Discontinuities in Arrhenius plots have been observed at temperatures that cause chilling injury in chilling-sensitive plants but do not occur in chilling-resistant plants (Markhart 1986). However, membrane changes may be secondary to other cellular alterations. For example, microtubules were shown to depolymerize when exposed to low temperatures (Carter and Wick 1984) and membrane protein conformation or lipid-protein interactions were more susceptible than lipid bilayers to low temperature (Yoshida 1984).

Low soil temperature reduced photosynthetic rates (Day et al 1989, Vapaavuori et al 1992, Folk et al 1995) and plant growth (Lopushinsky and Max 1990, Vapaayuori et al 1992, Folk et al 1995, Landhausser and Lieffers 1998) in various tree species. Folk et al (1995) found that low soil temperature reduced shoot gas exchange rate, induced shoot water stress and inhibited plant growth in yellow-cedar. Day et al (1989) showed that low soil temperature limited photosynthesis in *Pinus taeda*. Root growth and nitrogen absorption in both *Pinus sylvestris* and *Picea abies* were inhibited at root temperatures of 5 and 8°C (Vappavuori et al 1992). At a 5°C soil temperature, root and shoot growth and photosynthesis in *Pinus sylvestris* were limited (Ryyppo et al 1998). It is known that under cold soil conditions, sucker initiation in aspen is reduced (Zazada and Schier 1973, Johansson and Lundh 1988, Lavertu et al 1994) and that photosynthetic rate of *Populus tremuloides* is reduced at low soil temperature (Lawrence and Oechel 1983). Recently,

Landhausser and Lieffers (1998) found that low soil temperature (6°C) strongly restricted leaf area expansion, and increased root and shoot dry weights. In Douglas-fir, stomatal conductance of seedlings grown in cold soil (1.3°C) was 50 percent or less than those grown in warm soil (20°C) (Lopushinsky and Kaufmann 1984). Delucia (1986) reported that in Engelmann spruce seedlings, the stomatal conductance declined sharply below 8°C soil temperature. Kaufmann (1975) found that the resistance of water transport through Engelmann spruce did not reduce until 8°C of soil temperature. Plant water stress and limited plantation establishment have been attributed to low soil temperature during the spring planting season (Nambiar et al 1979, Grossnickle and Blake 1985, Vapaavuori et al 1992). Kaufmann (1975, 1977) studied the relationship between soil temperatures and water conductance through whole conifer plants. There are no reports the literature that root hydraulic conductance is related to the soil temperatures in woody species, while the relationship was reported on herbaceous plants (Markhart et al 1979a, 1990). Specifying water conductance as root hydraulic conductance may be more directly related to the effects of the soil temperatures. Additionally, previous experiments with woody plants were mainly designed to test the effects of low soil temperature on the survival and growth of the traditional economically important coniferous species. Although deciduous woody plants are often found in the same sites with coniferous trees, their responses to low soil temperatures may be different.

Root water absorption depends on the absorption area, root hydraulic conductivity and driving force for water movement:

$$Q = A\, L_p\, \Delta\psi_w$$

Where Q is the water flow ($cm^3\ sec^{-1}$),

A the absorption area (cm^2),

$\Delta\psi_w$ the water potential gradient (MPa),

L_p the root hydraulic conductivity ($cm^3\ cm^{-2}\ sec^{-1}\ MPa^{-1}$ or $cm\ sec^{-1}\ MPa^{-1}$).

New root growth is crucial for newly planted conifer species, and it can largely improve water uptake ability (Sands 1984, Grossnickle and Black 1985). However low soil

temperatures usually delay or restrict root growth of newly planted conifer seedlings (Lopushinsky and Kaufmann 1984, Delucia 1986, Lupushinsky and Max 1990). Lupushinsky and Max (1990) found that root growth started at 5°C or higher, its rate increasing with temperature to 20°C in several conifer species (*Pseudotsuga menziessi*, *Abies amabilis*, *Abis procera*, *Pinus contorta*, *Pinus ponderosa*). Both the expansion of the absorption area and root permeability may be affected by long-term low soil temperature conditions, while exposing root systems to low soil temperature for a short-term reduces water uptake mainly by combined low root water permeability and increased water viscosity (Kaufmann 1975). At low temperature, when membrane lipids are less fluid and membrane proteins are somewhat immobilized, the resistance of the plasma membrane to water flow is high (Markhart 1986). Adaptation and acclimation to low temperature generally involves a shift to more unsaturated fatty acids, which increases the fluidity of these membranes at low temperature (Markhart et al 1980, Lambers et al 1998). In addition, it should be noted that temperature effects on the water channels via gating, i.e. conformational changes of the protein caused by temperature, may conceal the actual temperature dependence of water flow (Tyerman et al 1999).

Low air temperature can also damage trees in many ways: foliar injury, bud killing, stem girdling or patch killing of the cambium, frost cracking of stems and embolism formation induced by freeze-thaw cycles, etc. (Tyree and Sperry 1989, Kimmins 1997). Intracellular and intercellular ice formation is one of the causes of freezing damage. However spontaneous intracellular ice formation generally does not occur (Sakai and Larcher 1987). It is clear that the plasma membrane plays a central role in cellular behavior during a freeze-thaw cycle, and disturbance of the semipermeable characteristic or lysis of the plasma membrane and/or the tonoplast is a primary cause of freezing injury (Ziegler and Kandler 1980, Sakai and Larcher 1987). Species from different regions respond differentially to low temperatures. Mangrove in the tropics exposed to 2 to 4°C suffered from severe damage and died (Markley et al 1982), while conifers in boreal or alpine regions can survive freezing to –70°C or below (Sakai and Larcher 1987).

In the boreal forest, detailed studies on damage to forest species in harsh winter are concerned with winter desiccation and photoinhibition of photosynthesis. If the water in the soil remains frozen and/or the conducting conduits of stems are blocked by ice or embolism, the water balance of the plant becomes negative because transpiration does not entirely cease in winter; the resulting disturbances are termed winter desiccation (Sakai and Larcher 1987). *Thuja occidentalis* survives freezing to –70°C , but is very sensitive to winter desiccation (Sakai 1970). Winter desiccation of needles is also thought to limit tree growth and survival within alpine timberline ecotones (Sowell et al 1996). The freeze-thaw induced embolism formation blocks water supply to needles in winter (Sperry 1993).

Low temperature with high light intensity inhibits photosynthesis of plants. This phenomenon is called photoinhibition (Oquist and Martin 1980, Sakai and Larcher 1987). Sustained depressions in photosystem II efficiency are commonly interpreted as photoinhibition. In mid-winter, cold-induced photoinhibition (indicated by loss of quantum yield) increased with an increase in exposure to high irradiances, with quantum yield in the most exposed leaves averaging 51% less than that expected in fully functional leaves of *Eucalyptus pauciflora* (Ball et al 1991). The occurrence of photoinhibition has been widely reported in evergreen forest species (Weger et al 1993, Adams and Demmig Adams 1994, Krol et al 1995, Ottander et al 1995).

3. Water uptake and ascent

Water movement occurs along gradients of decreasing free energy, often expressed as differences in water potential (ψ_w). The water potential in the atmosphere is almost always lower than that in soil, therefore water movement in plants is primarily from soil to air. In moist soil, the rate of water absorption is controlled primarily by two factors: the rate of transpiration, because it largely controls ψ_w in the root xylem, and the efficiency of root systems as absorbing surfaces (Kozlowski and Pallardy 1997).

There are two absorption processes which generate the ψ_w gradients: osmotically driven absorption and passive absorption. Osmotically driven absorption occurs in slowly transpiring plants. In this case, the roots of plants function as osmometers. Actively absorbed ions accumulate in the root xylem sap which lowers the ψ_π (osmotic potential) and ψ_w by which water is driven into the roots from the soil. In transpiring plants, the ψ_w gradients primarily originate in the transpiring shoots. The roots become passive absorbing organs through which water is pulled in by mass flow generated in the transpiring shoots. There is no evidence of any direct active water transport that consumes energy to transport water molecules against the electrochemical gradient in plants.

The pathway of radial water movement in roots has long been a subject of debate. Water crosses the epidermis, cortex, endodermis, and sometimes also the exodermis before reaching the stele. The anatomical complexity of the roots makes complex the water movement through it. Steudle (1994) proposed a composite transport model of radial water flow through the roots. According to the composite transport model, there are different parallel pathways for water transport in roots: apoplastic, symplastic and transcellular paths. The apoplastic path is through the cell walls and intercellular space. The symplastic path is from cell to cell in the cytoplasmic continuum bridged by plasmodesmata between adjacent cells. In the transcellular path, water crosses cell membranes. The latter two paths cannot be experimentally separated and the two are often referred to as the cell-to-cell or symplastic pathway.

Water can move through both apoplastic and symplastic paths until it reaches the endodermis if the roots do not possess an exodermis. Water can also be exchanged between the two pathways based on their hydraulic conductivities. The Casparian strips block water movement through the walls of endodermal cells and thus force water into the symplast. The exodermis, found in relatively few species, provides a similar barrier to apoplastic flow of water. In other words, the apoplastic pathway cannot by itself facilitate water movement from soil to the stele of roots. Therefore water flow through roots

becomes symplastic (Steudle and Jeschke 1983, Boyer 1985). However, the endodermis does not always form a permanent impermeable barrier to water and solutes (Steudle and Peterson 1998). When young roots develop in the pericycle and push out through the endodermis, gaps are produced through which water and solutes can freely enter (Skinner and Radin 1994). Water travels along symplastic and apoplastic paths through the root. If the apoplast is the main path for water flow, it must have a low resistance compared to the adjacent symplast. On the other hand, if the apoplast has high water flow resistance, water would be forced through the symplast or from cell to cell across cell wall and plasmalemma. A comparison of single cell and whole-root kinetics indicated that apoplastic flow could not predominate (Steudle and Jeschke 1983). Water likely moves from cell to cell and through the apoplastic route. In addition, water must eventually enter the symplast at the endodermis, where the Casparian band prevents further transport through the apoplast. Therefore, the apoplast alone could not provide the principal pathway for water transport through the roots from outside to the stele. In healthy root systems, only 1% of the water bypasses through the Casparian band (Hanson et al 1985, Skinner and Radin 1994). In some species, the exodermis also forces water to travel through the plasma membrane (Peterson 1988). Both endodermis and exodermis may have evolved to control ion absorption, which maintains ions and the osmotic balance in plants. At the same time, they also change the water flow pathway. It is presently believed that the exodermis and endodermis are the rate-limiting barriers to water transport and force water to travel through the plasma membrane. It is important to recall at this point that water can move either by bulk flow or by diffusion through the membrane (Boyer 1985). Water channels in the cell membranes facilitate the water bulk flow (see section 6, water channel proteins).

The existence of tall land plants became possible only after the plants had evolved a vascular system that permitted rapid movement of water to the transpiring shoots. Water moves upward in the xylem through the roots and stems and to the leaves. So far, despite some weak points, the cohesion-tension theory is the only theory that can explain how absorption and transpiration are effectively coupled together (Kozlowski and Pallardy

1997). Dixon and Joly (1895) pointed out that water confined in small tubes, such as the xylem elements, has a very high cohesive force and can be subjected to high tension.

Water may pass through several living cells before reaching the evaporating surfaces in shoots. Water movement through leaves seems to be largely apoplastic without endodermis. However, there is evidence that water movement out of the leaf xylem is primarily symplastic (Canny 1990).

4. Root hydraulic conductance

Water movement is governed by two fundamental factors: the driving force and the hydraulic conductance of the flow pathway. Major hydraulic resistance in plants is within the roots (Kramer 1983). Accordingly, root hydraulic conductance plays an important role in controlling water uptake. However, root conductance is affected by many environmental factors such as drought, temperature, anoxia and hypoxia (in situations of flooding or soil compaction), nutrient deficiency, etc. (for review, see Boyer 1985, Passioura 1988c, Markhart and Smit 1990, Lambers et al 1998). Understanding the properties of root hydraulic conductance is essential to understanding the impact of the soil environment on water uptake and plant water relations.

The general principle of measuring root hydraulic conductance is similar to the measurement of membrane permeability. For a membrane, a water potential gradient is established across the membrane and the flux of the water is measured. Then, conductance of the membrane is calculated using the following equation.

$$Q = \Delta\psi_w L$$

Where Q is the water flow ($cm^3\ sec^{-1}$),

$\Delta\psi_w$ the water potential gradient (MPa), and

L the hydraulic conductance ($cm^3\ sec^{-1}\ MPa^{-1}$).

If the surface area of the membrane is known, then the hydraulic conductance can be converted to a hydraulic conductivity, L_p ($cm^3\ cm^{-2}\ sec^{-1}\ MPa^{-1}$). The above equation holds true only when the membrane is ideally differentially permeable to water and solutes. If not, there is an interaction between hydrostatic potential and osmotic potential (Dalton et al 1975, Fiscus 1975), then the equation should be written as:

$$Q = L\ (\Delta\psi_p + \sigma\Delta\psi_s)$$

Where σ is the reflection coefficient expressing the ability of the membrane to reflect solute (varies between 1 for a fully reflective membrane and 0 for a non-reflective membrane).

Generally, metabolites and inorganic ions cross cell membranes slowly, and therefore, σ approaches 1. At low fluxes, water influx may dilute solute concentration inside, which could be attributed entirely to this interaction between water and solute transport. At high rates of water flow, further changes in solute concentrations are negligible in the cell or the xylem (Dalton et al 1975, Fiscus 1975). Therefore, they showed that the conductance to water could be assessed most easily at high fluxes.

For plant tissues or organs, certain approximations need to be made to accommodate membrane or cell phenomena. L is the average conductance of the path between the source and the tissue or organ and $\Delta\psi_w$ refers to the average water potential difference between the water source and the tissue rather than across the membrane of a cell.

Root hydraulic conductance is determined by measuring the water flow rates triggered by a water potential gradient. The methods vary mainly in how the water potential gradient is established. The popular methods include suction, osmotica, hydrostatic pressure and pressure probe. Suction is creating a hydrostatic potential gradient by vacuum. The maximum hydrostatic pressure gradient produced by a vacuum is 70 KPa, that is the range where the interaction of hydrostatic and osmotic driving forces is most pronounced (Fiscus 1975). Osmotic changes create an osmotic potential gradient. However, some osmotica penetrate tissue slowly, so that not all cells are affected similarly by the

solution. Furthermore, solute exchange occurs between the cell and the solution, and the forces affecting water movement may then be altered (Dainty 1963). The pressure probe is an improved method that takes advantage of single cell measurements and water relations of whole root systems (Zhu and Steudle 1991, Steudle and Meshcheryakov 1996). However, the apparatus is expensive and the operation is quite complicated. Besides, a series of calculations based on assumptions must be used in this method (Hüsken et al 1978). Hydrostatic pressure applies a positive pressure to detopped root systems, which forces water up the root. The positive pressure is opposite to the natural way in which a pressure gradient is produced by transpirational pull, however the method adequately measures the natural properties of water transport through the root systems (Markhart and Smit 1990). Hydrostatic pressures applied are similar in magnitude to the tensions in the xylem solution occurring naturally, and their produced flow rates are comparable to those in rapidly transpiring plants (Boyer 1985). Therefore the conductance properties of the roots are probably similar to those the intact system.

5. Water relations in deciduous species

Deciduous species are those shedding leaves at a certain season. The deciduous habit allows woody plants to lose their leaves and become relatively inactive physiologically during periods of drought, extreme heat or cold. The leaf-dropping in winter may be related to the unavailability of water, which is a consequence of soil temperatures below the freezing point. These conditions delay the movement of soil water to the roots (Raven et al 1999). Deciduous species differ from evergreen ones in the nature of their water consumption. In winter, temperate deciduous plants are leafless and their evapotranspiration is greatly reduced (Kramer 1983). Compared with evergreens, deciduous trees generally possess higher transpiration rates in the summer (Kramer and Boyer 1995, Lambers et al 1998). However the annual transpiration rate favors evergreens. Kaufmann (1985) reported that in the subalpine forests of Colorado, the annual transpiration rates of three conifers (lodgepole pine, subalpine fir and Engelmann spruce) were higher than those of aspen.

Seasonal studies on deciduous temperate species have shown that during the winter up to 100% of the hydraulic conductivity can be eliminated by freezing-induced xylem embolism (Sperry et al 1987, 1988, Cochard and Tyree 1990). The magnitude of the freezing induced embolism is much higher in the deciduous than in evergreen species (Sperry 1993). Vulnerability to embolism formation in winter is related to the diameter of xylem elements in the stem and the root. According to Sperry and Sullivan (1992), the large earlywood vessels of oaks are most susceptible to embolism formation, the small vessels of poplar and birch are intermediate, and the tracheids of conifers are least susceptible. However, susceptibility to embolism formation under water stress in summer is not correlated with whether a conduit is a hardwood vessel or a softwood tracheid (Tyree and Sperry 1989, Sperry 1993). Deciduous species possess more effective conducting systems than evergreen plants. A more efficient xylem may be a prerequisite for sustaining the relatively short-lived, metabolically more active leaves of deciduous species compared with that required for long-lived, metabolically less active leaves of evergreen species (Kolb and Davis 1994, Larcher 1995, Tognetti et al 1999). Enhanced efficiency of hydraulic conductivity may permit increased transpiration rates during the spring and the summer, thus facilitating rapid growth and high rates of photosynthesis in deciduous species.

6. Water channel proteins

For a long time, it was thought that water transport into and out of the plant cells was accomplished by diffusion directly through the lipid bilayer. This view was challenged by the recent discovery of aquaporins, which are protein molecules forming channels in the cell membranes (Chrispeels and Maurel 1994). After their discovery in the plasma membranes of animal cells (Preston et al 1992), water-channel proteins were also found in the tonoplast (Maurel et al 1993), and then in plant plasma membranes (Daniels et al 1994, Kammerloher et al 1994). Aquaporins are part of an ancient family of channel proteins--major intrinsic proteins (MIP) that are integral membrane ingredients. There

exist three subclasses of MIP in plants: the tonoplast intrinsic proteins (TIP), the plasma intrinsic proteins (PIP) and the legume nodule protein (NOD26) (Maurel 1997). The polypeptide chains of all the MIPs span the membrane six times and have amino and carboxyl termini which face the cytoplasm (Preston et al 1994). Water channels confer an increase in membrane water permeability, relative to the lipid bilayer, of one to two orders of magnitude (Tyerman et al 1999). Water permeability across lipid vesicles is about 10×10^{-6} m s^{-1} (Finkelstein, 1987, Lande et al 1995), while typical values for membranes with water channels are between 100-200 $\times 10^{-6}$ m s^{-1} (Zeidel et al 1994).

In addition to the direct molecular evidence, the existence of water channels can be demonstrated from the properties of water channel function. Several criteria have been used to infer the presence of water transporting channels in cell membranes. These include a high ratio of osmotic to diffusional water permeability ($P_f/P_d > 1$), low Arrhenius activation energy ($E_a < 6$ kcal·mol^{-1}) for water transport, and its reversible inhibition by mercury sulfhydryl reagents (Chrispeels and Agre, 1994, Verkman et al, 1996, Maurel et al, 1997). Diffusional transport of water is through the lipid bilayer. The lipid bilayer packs more tightly at lower temperature, making the transport of water highly temperature-dependent (Chrispeels and Agre, 1994). Water movement through the lipid bilayer would need to surmount the high-energy barrier of water partitioning into the hydrophobic lipid phase. Therefore, in diffusional transport, E_a is usually high, above 10 kcal mol^{-1} (Macey, 1984). Osmotic water transport is via water channel proteins (aquaporins). Water transport across a channel does not have to overcome a large energy barrier. The temperature dependence of the water osmotic permeability should be comparable to that for the viscosity of water. The presence of pores facilitates bulk water flow across the membrane. It is generally acknowledged that the osmotic transport of water possesses less temperature dependence, and lower E_a (< 6 kcal mol^{-1}) than diffusional transport (Finkelstein, 1987, Chrispeels and Agre, 1994). It is noteworthy that temperature dependence may be mediated via the channel gating (Tyerman et al 1999). The osmotic transport is characteristically inhibited by mercurial reagents, which react with sulfhydryl groups in channel proteins and result in closure of the water channels.

This closure inhibits osmotic transport and increases E_a to the level of diffusional permeability (Macey, 1984). An inhibition of water transport by mercury was reported in cell membranes isolated from higher plants (Niemietz and Tyerman, 1997, Maurel et al, 1997) and in whole root systems (Maggio and Joly, 1995, Carvajal et al, 1996). However, caution should be taken with mercury sulfhydryl reagents application because the substances are general metabolic inhibitors (Schütz and Tyerman 1997).

7. Stomatal response to hydraulic or chemical signals

Transpiration is an inevitable consequence of photosynthesis (Salisbury and Ross 1985, Lambers et al 1998). In the absence of spatial selectivity for the diffusion of water and carbon dioxide, stomata provide a temporal adaptation. Stomata close when water is limiting and open under conditions favoring photosynthesis, thus preventing excessive water loss. Stomatal movements depend on turgor regulation in the guard cells. When an increase in the osmotic concentration of the guard cells drives water uptake and the guard cell swelling results in a separation of the two guard cells, stomata open because of the radial reinforcement of the guard cell wall. Conversely, stomatal closure occurs when the loss of solutes from the guard cells results in water and turgor loss and guard cell deflation (Raschke 1987, Assmann and Shimazaki 1999).

The steepest water potential gradient in the soil-plant-atmosphere continuum occurs at the leaf surface, which indicates that stomata are the major control point for plant water relations (Lambers et al 1998). Irradiance, the CO_2 concentration, humidity of the air, and water availability in soil affect stomatal movements (Zeiger et al 1987, Schulze 1986). Among these, stomatal response to reduced water availability in the soil has been often debated (Kramer 1988, Passioura 1988a, Boyer 1989). The debate has mainly focused on the relative importance of hydraulic versus chemical signals to trigger stomatal movement under water stress conditions. Water transport takes place in a soil-plant-air continuum that is interconnected by a continuous film of liquid water. This water can provide a route for hydraulic and chemical signal communication. As previously

mentioned, stomatal aperture is regulated through osmotic changes in the guard cells which mediate the flux of solutes on sensing signal(s), regardless of whether they are hydraulic or chemical in nature.

Water movement is determined by the water potential gradient. Water potential in the guard cells is usually much higher (less negative) than that in the air (Raschke 1987, Lambers et al 1998). The guard cells simultaneously lose water to the air and uptake water from their surrounding epidermal cells cell walls and around the substomatal chamber. In order to keep the shape of the guard cells and the stomatal aperture, the loss and the uptake of water need to maintain a relatively dynamic balance. However, excessive vapor pressure deficits or severe shortage of water supply cause water to be lost more quickly than it can be absorbed.

Stomata exert, to a certain extent, control plant water relations, by either feedforward (Cowan 1977, Farquhar 1978, Franks et al 1997) or feedback responses (Raschke and Kühl 1969, Monteith 1995, Lambers et al 1998). In the feedback response, the stomata directly respond to leaf water potential (Monteith 1995, Bunce 1996). The guard cells sense leaf water potential by measuring changes in relative volume or relative turgor of the cells. The hydraulic signal triggers an unknown mechanism, which reduces the solutes in the guard cells and induces stomatal closure when the water potential is low enough (Raschke 1987). In the feedforward response, stomatal conductance declines before any adverse effects of water shortage arise in the leaves. Therefore, it has been argued that it must be chemical messages, rather than a hydraulic signal, which initiates osmotic regulation in the guard cells. Works by Gollan et al (1986) and Passioura (1988b) supported this contention. They used a pressure vessel placed around the roots of a seedling growing in drying soil. As the soil dried out, the hydrostatic pressure on the roots was increased so as to maintain shoot water potential similar to that of well-watered plants. Despite having the same leaf water status as the control plants, the treated plants showed reductions in leaf conductance similar to those of plants in drying soil outside a pressure chamber. Another line of evidence came from split root experiments (Zhang et al

1987) in which root systems of individual plants were split by half and grown in two containers. One container was well watered while water was withheld from the other one. Without any significant leaf water deficit, stomatal conductance was reduced. The pressurizing or the split root experiments demonstrated that some chemical message, traveling from roots to shoots, initiated stomatal response. The phytohormone, abscisic acid (ABA), was proposed as the predominant root to shoot messenger (Davies and Zhang 1991, Davies et al 1994).

The feedforward response of stomata to root or soil water potential seems well established, although in other species, stomatal control is hydraulic and no chemical signal from the roots appears to be involved (Fuchs and Livingston 1996). However, as Kramer (1988) pointed out, such conditions of low soil water potential and artificially high leaf water potential do not often happen in nature. Also, environmental conditions may maximize the role of roots and minimize that of leaves in stomatal control. In addition, stomatal response to air vapor pressure deficits can not be well explained by the theory of chemical signals (Monteith 1995, Bunce 1996).

8. Role of ABA in water relations

It has become clear that ABA can act as a second messenger at the molecular, cellular and whole plant levels, where it transduces environmental effects into biological responses. It seems, therefore, that the stress responses of plants cannot be understood without some consideration of a role for ABA (Davies and Jones 1991). Protection from dehydration damage is one important role of the hormone. Little and Eidt (1968) first noted that applying ABA to woody plants reduced transpiration. Soon, the effect was demonstrated to be due to stomatal closure (Mittelheuser and van Steveninck 1969). Wright and Hiron (1969) found that wilting induced an increase in ABA in detached wheat leaves. The observations led to the hypothesis that ABA is a messenger between the mesophyll cells and the epidermis. Since then, numerous reports have shown that ABA can have a

powerful effect on the stomatal conductance in many species (for examples see Kriedemann et al 1972, Raschke and Hedrich 1985, Mansfield et al 1990).

A reduction of leaf turgor induces a rapid increase in the concentration of ABA in the leaf, as well as stomatal closure (Zeevaart 1980), although the increase was frequently detected only after stomatal closure (Pierce and Raschke 1980). It has been established that ABA is the signal molecule responsible for closing stomata during dehydration. There are two steps in the process (Cornish and Radin 1990). In the first step, ABA is rapidly released from the mesophyll compartments to the apoplast, (the primary site of action of ABA is the outer surface of the guard cells (Hartung 1983)). This step occurs without any significant overall increase of ABA in the leaves. The response can be promoted by a small change in leaf water status (Hartung and Davies 1991). The second step involves ABA production, which occurs after the closure has begun and reinforces the effect of stored ABA. The role of ABA in closing the stomata is to decrease the turgor in the guard cells by inhibiting the K^+ uptake and stimulating K^+ and Cl^- efflux (Raschke 1987, Assmann and Shimazaki 1999). Ca^{2+} also participates in the process of ABA action (Assmann and Shimazaki 1999).

There is also evidence that when a plant endures water stress in drying soil, ABA is synthesized in the roots and translocated to the leaf through the transpiration stream (Cornish and Zeevaart 1985, Zhang et al 1987). These results support the hypothesis that ABA produced in stressed roots may act as a chemical signal to the shoot, initiating stomatal closure before any bulk changes in water potential or turgor in leaves occur (Davies and Zhang 1991). Subsequently, numerous reports showed that the concentration of ABA in xylem sap is tightly correlated with stomatal aperture (Zhang and Davies 1990, Wartinger et al 1990, Tardieu et al 1992, Khalil and Grace 1993, Tardieu et al 1996).

Since root systems produce ABA and fairly high concentrations of ABA exists in the rhizosphere soil (Hartung et al 1996), the effect of ABA on root water uptake is also

potentially important. While there is no doubt that stomata close in response to ABA, the response of root water transport to ABA seems less clear. Glinka (1977, 1980) showed that exogenous application with high ABA concentrations increased the rate of water movement through sunflower roots. However, these increases could not be confirmed when high flow rates were used to minimize the effects of high solute concentrations (Markhart et al 1979b, Fiscus 1981, Davies et al 1982). Pitman and Wellfare (1978) were also unable to find long-term effects on root water transport over a range of concentrations of ABA. The variable effects of ABA on root hydraulic conductance were attributed to a possible increase in hydraulic conductance by ABA when the root flow rate was low and a decrease when the rate was high (Fiscus 1981, Davies et al 1982). Recent reports of increases in root hydraulic conductance by ABA were also conducted at relatively low water flow rates (Freundl et al 1998, 2000, Quintero et al 1999).

Figure 1-1. Shaded area indicates the distribution of aspen (*Populus tremuloides*) in North America (from Peterson and Peterson 1992).

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CHAPTER TWO[1]

Root Water Flow and Growth of Aspen (*Populus tremuloides*) at Low Root Temperatures

ABSTRACT

Effects of root zone temperature on growth, shoot water relations and root water flow were studied in one-year-old aspen (*Populus tremuloides*) seedlings. The seedlings were grown in solution culture and exposed to day/night air temperature of 22°C/16°C and solution temperatures of 5, 10, or 20°C for 28 days after bud flush. Root growth was completely inhibited at 5°C and 97% inhibited at 10°C compared with root growth at 20°C. Both the 5 and 10°C treatments severely reduced shoot growth, leaf size, and total leaf area. Root water flow was inhibited at 5 and 10°C temperature treatments and it was positively correlated with new root growth. However, when seedlings were grown for 28 days at 5°C and root water flow was measured at 20°C, there was an increase in flow rate of a similar magnitude to the decrease observed when root water flow of seedlings in the 20°C treatment was measured at 5°C. Low root water flow at the 5 and 10°C temperature treatments restricted stomatal conductance, net assimilation, and shoot water potentials. Root water flow was positively correlated with leaf size, total leaf area, and shoot length. Transferring seedlings from 5 to 20°C for 24 hours, significantly increased root water flow, shoot water potential, and net photosynthesis, whereas transferring seedlings from 10 to 20°C resulted only in a slightly increased shoot water potential. However, a change from 20 to 5°C caused drastic reduction in root water flow, stomatal conductance, and net photosynthesis, whereas shoot water potential decreased only slightly.

Keywords: boreal forest, water balance, low soil temperature, root growth

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[1] A version of this chapter has been published. Wan X, Landhäusser SM, Zwiazek JJ, Lieffers VJ 1999 Tree Physiology 19, 879-884.

INTRODUCTION

In northern forests, air and soil temperatures are key factors limiting tree growth (Tryon and Chapin 1983, Bonan 1992). Although the impacts of extremely low air temperatures on trees are well described, few studies have examined impacts on tree responses to low soil temperatures during the growing season. In boreal forests, low soil temperatures in summer are common on sites with a thick layer of organic matter or heavy cover of grass thatch (Viereck 1970, Hogg and Lieffers 1991). Low soil temperatures have been shown to inhibit shoot and leaf growth (Landhäusser and Lieffers 1998), root growth (Folk et al 1995) and photosynthesis (Vapaavuori et al 1992, Folk et al 1995) in various tree species. Reduced nutrient cycling has been invoked as a mechanism to explain reduced growth rates in cold soils (Pastor et al 1987, Paré et al 1993), but few studies have examined the effect of cold soils on water relations of trees during the growing season. Similarly, the role of root hydraulic conductivity in controlling tree water relations and growth has received little attention. In cold soil temperature sensitive species, a reduction in water uptake can occur in cold soils as a result of decreased root permeability and increased water viscosity (Kaufmann 1975). It has been also reported that the type and amount of roots can affect root hydraulic conductivity (Fiscus and Markhart 1979, Rüdinger et al 1990). Therefore, it is possible that the roots of trees exposed to relatively high air temperatures and cold soils may be unable to conduct water at high enough rates to meet the transpirational demands of the shoots. This upset in water balance could result in xylem cavitation and affect tree survival.

The objective of the present study was to examine water relations and growth in aspen (*Populus tremuloides*) exposed to low root temperature and develop a method to measure root water flow at different temperatures. In our experiments, we focused on the early growth stages of *P. tremuloides* seedlings following winter dormancy. We hypothesised that the reported growth inhibition of *P. tremuloides* in cold soils is the result of a water imbalance caused by reduced root water flow. We selected *P. tremuloides* for this study

because of its economic importance, slow growth on sites with thick organic layers (Landhäusser and Lieffers 1998) and numerous cases of early die-back on mature stands. This species exhibits reduced growth at temperatures <10°C in well fertilised conditions (Landhäusser and Lieffers 1998) and thus seems to be an ideal candidate for examination of the effects of low root temperatures on root hydraulic conductivity and growth.

MATERIALS AND METHODS

Plant material and treatments

We used one-year old bareroot *P. tremuloides* seedlings grown by a local nursery from seed that had been collected at Drayton Valley, Alberta, Canada. The frozen, dormant seedlings were thawed in a cold room at 4°C for three days. Roots of 150 seedlings were gently washed and the seedlings were grown for two weeks in a growth chamber in aerated half-strength modified Hoagland's solution (Epstein 1972) set at 5°C. The growth chamber provided a 18/6 light/dark cycle with 22/16 °C day/night temperature, 60% relative humidity, and 400 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at the seedling level.

After two weeks, the flushed seedlings were divided randomly into three groups and transferred to pans (52×35×20 cm) containing aerated half-strength modified Hoagland's nutrient solutions that were maintained at 5, 10, or 20°C by three circulating cooling systems that delivered nutrient solutions of the desired temperature, from insulated 50-L reservoirs (adapted from Landhäusser et al. 1996).

The experimental design was factorial with root temperature and days of treatment as the fixed main effects. Differences among bench positions were minimised by regularly moving plants at random to different positions within the container.

The experiment consisted of three parts. The first part tested the development of the water balance in *P. tremuloides* seedlings grown at different soil temperatures over a time

period of one month. The measurements were conducted on days 1, 2, 7, 14, and 28 following the transfer to 5, 10, or 20°C. On each measurement day, leaf stomatal conductance, shoot water potential, root water flow, leaf area, leaf dry weight, shoot height, root dry weight, and new root length were measured in seven seedlings from each of the three root temperatures.

The second part examined the short-term response of the temperature-adapted root systems to a change in temperature. Seedlings that had been grown at 20°C root temperature for 28 days were transferred to 5°C, and seedlings grown at 5 and 10°C were transferred to 20°C. After 24 hours, leaf stomatal conductance, shoot water potential, root water flow and photosynthesis were measured and compared with the control plants that remained in their original temperature treatments.

In the third part, roots of seedlings subjected for 28 days to different temperature treatments were immersed in distilled water at 5, 10, and 20°C and root water flow was measured at these temperatures as described below.

Measurements

All measurements were conducted between 3 to 5 hours after the start of the photoperiod. Leaf stomatal conductance (g_S) was measured with a steady-state porometer (LI-600, Li-Cor Inc., Lincoln, NE), water potentials on whole shoots were measured with a Scholander pressure chamber (Scholander et al 1965) and projected leaf areas were determined with a leaf area meter (LI-3100, Li-Cor, Inc.). New root length was digitised by scanning (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA) and dry weights were determined after freeze-drying for 72 hours.

Root water flow (Q_V) was determined as a steady-state flow rate of the whole root system. The flow rate was measured by the hydrostatic pressure method as previously described (Fiscus 1981, Sailerova and Zwiazek 1996) with some modifications. A glass cylinder was inserted in a pressure chamber (PMS Instruments, Corvallis, OR) and filled

with distilled water. The seedling's stem was cut about 20 mm above the root collar and the root system was immersed in distilled water and surrounded by a copper coil that was connected to a circulating cooler system (Haake F3, Berlin, Germany) to maintain the desired root temperature (± 0.1°C). A pressure of 0.4 MPa was gradually applied and maintained during the measurements. A graduated pipette was attached with a short piece of rubber tubing to the stem protruding through the stopper in a pressure chamber. Root flow rates were monitored for linearity for at least 30 min. For comparison, values of Q_V were expressed on a per seedling basis (μl H_2O min^{-1} $seedling^{-1}$) and on a per unit length of new roots basis (μl H_2O min^{-1} cm^{-1}).

Data analysis

After logarithm transformation of the total leaf area, new root length, and root water flow rate, all response variables met the assumption of normal distribution and homogeneity of variances. To test for treatment effects, analysis of variance procedures and least significant difference multiple comparisons were performed with general linear models available in SAS 6.11 (SAS Institute Inc., Cary, NC). Two-way ANOVA was for date and temperature treatment. For each of date, one way ANOVA was performed. Correlation analyses between water flow and leaf area or shoot height were performed. The significance levels were set at $\alpha = 0.05$. Data from the short-term temperature treatment were analysed by a two tailed t-test.

RESULTS

Part I

Growth response

Leaf size and total leaf area per seedling did not change during the initial 7 days (Figures 2-1a,b). After 14 and 28 days, the mean leaf area of seedlings in the 20°C root temperature treatment was several-fold higher than that of seedlings in the lower root temperature treatments (p=0.0001, one way ANOVA) (Figure 2-1a). Similarly, the total leaf area per seedling in the 20°C root temperature treatment was slightly higher on day

14 (p=0.0101) compared to that of seedlings in the two other root temperatures; however, after 28 days total leaf area had increased to 476 cm^2 in seedlings at 20°C compared to 32 and 55 cm^2 in seedlings at 5 and 10°C, respectively (p=0.0001) (Figure 2-1b).

Shoot height followed a similar pattern to leaf area development. Shoots of seedlings exposed to 20°C root temperature elongated on average 19.2 cm after 28 days compared to approximately 7.2 cm and 3.6 cm at 10°C and 5°C, respectively (p=0.0001) (Figure 2-1c)

Initiation and growth of new roots were affected by root temperature (Figure 2-1d). At 5°C, the initiation of new roots was almost totally inhibited over the experimental period. Seedlings with roots exposed to 10°C produced an average of 28 cm of new roots per seedling during the 4 weeks of treatment, whereas seedlings subjected to a root temperature of 20°C had 976 cm of new roots per seedling (p=0.0001) (Figure 2-1d).

Water relations and gas exchange

There was no difference in stomatal conductance (g_S) among the three root temperature treatments after seven days. After 14 days, plants growing at a root temperature of 20°C had g_S of 181 mmol m^{-2} s^{-1} compared with 25 mmol m^{-2} s^{-1} for plants in the 5°C treatment (p=0.0001) (Figure 2-2a). Stomatal conductance of plants in the 5 and 10°C root temperature treatments remained low throughout the experiment; however, on day 28, the 10°C-treated seedlings had significantly higher g_S compared with seedlings in the 5°C treatment (p=0.0001) (Figure 2-2a).

Shoot water potentials (ψ_W) of seedlings with roots exposed to 10 and 20°C were significantly higher than those of seedlings with roots exposed to 5°C after only one day in the temperature treatments (p=0.0067) (Figure 2-2b). Shoot water potentials of seedlings in the 20°C treatment declined for the next several days and then increased to –0.8 MPa on days 14 and 28. In the 10°C treatment, ψ_W declined to –2.0 MPa by day 7

and then increased to –1.5 MPa by day 28, whereas ψ_w in 5°C-treated plants declined to –2.2 MPa by day 28 (Figure 2-2b).

Root water flow (Q_v) per seedling was similar in all plants during the first 2 days of temperature treatments (Figure 2-2c). However, from day 7 on, Q_v at 20°C was higher than at 5 and 10°C (p=0.01). On day 14, Q_v values in the 20°C-treated seedlings were about 20-fold higher compared with those of seedlings in the other treatments. On day 28, Q_v in the 20°C-treated seedlings was about 1416 µl min^{-1} $seedling^{-1}$ compared with 114 and 5 µl min^{-1} $seedling^{-1}$ in the 10 and 5°C treatments, respectively (Figure 2-2c).

While root water flow (Q_v) per plant increased in the 10 and 20°C treatment over the time period of 28 days, Q_v per cm of newly grown roots at the 20°C treatment increased from 0 to 7.7 µl min^{-1} cm^{-1} at day 7 (p<0.0001); however, Q_v decreased again on day 14 to 0.6 µl min^{-1} cm^{-1} (p<0.0001) (Figure 2-2d). On day 28, Q_v per cm of newly grown roots significantly increased to 1.5 µl min^{-1} cm^{-1} (p=0.0009) (Figure 2-2d). In the 10°C treatment, Q_v based on the unit length of new roots increased from 0 to 10.3 µl min^{-1} cm^{-1} (p<0.0001) only on day 28. Root water flow per cm of newly grown roots on day 7 in the 20°C treatment was not statistically different from Q_v measured on day 28 at the 10°C treatment (p=0.4335). Root water flow per unit length of new roots could not be measured in seedlings grown at 5°C since the seedlings did not produce new roots over the experimental period (Figure 2-2d).

Part II

When seedlings which had grown for 28 days at a root temperature of 5°C were transferred for one day to 20°C, Q_v increased by 3.5 times (p=0.0002) whereas ψ_w about doubled (p=0.0003) (Figures 2-3c,d). When seedlings were transferred from 10 to 20°C ψ_w showed a significant increase (p=0.0234), but Q_v did not change (p=0.669) (Figures 2-3c,d). Only plants transferred from 5 to 20°C showed an increase in net assimilation (p=0.0001) but g_s remained unchanged (p=0.113) (Figures 2-3a,b). A dramatic decline in Q_v (p=0.0002), g_s (p=0.0017), and net assimilation (p=0.0066) but only slightly in ψ_w

(p=0.038) was observed only 24 hr. after transferring seedlings from 20 to 5°C (Figures 2-3a,b,c,d).

Part III

The Q_v values of seedlings grown at a specific root temperature for 28 days was measured at the three respective temperatures. Root water flow values showed a response within 30 minutes of transferring the seedling to the new temperature. Thus Q_v of seedlings grown at 5°C increased within 30 min from 5 to 9 and 15 µl min^{-1} $seedling^{-1}$ when they were exposed to 10 and 20°C, respectively (Figure 2-4). Seedlings grown at 10°C did not show a significant increase or decrease in Q_v when exposed over a 30 min period to 20 or 5°C, respectively. However, in seedlings grown at 20°C root temperatures, Q_v significantly decreased from 1416 to 623 and 473 µl min^{-1} $seedling^{-1}$ when seedlings were transferred to 10 and 5°C, respectively (Figure 2-4).

DISCUSSION

The cause of reduced growth of aspen in cool soils appears to be related to reduced water flow in roots (Q_v). The re-establishment of the cold-stored bareroot seedlings was accompanied by massive growth of new roots in the 20°C temperatures but only very slow growth of new roots at 10°C. These new roots had a large impact on Q_v when expressed on a whole seedling and on a unit length basis. Very young new root tips seemed to have the highest amount of Q_v on a unit length basis. When roots matured, Q_v was reduced probably due to a higher proportion of more mature suberized root parts in the new root system as has been suggested by Rüdinger et al (1994). The importance of root age on Q_v has also been shown in *Phaseolus vulgaris* (Fiscus and Markhart 1979). Root water flow again slowly increased towards the end of the experimental period at 20°C. This could be due to an increased amount of root branching which produced a higher ratio of new roots tips to the overall new root length. The start of a similar pattern in Q_v could be detected in seedlings growing in the 10°C treatment. Here Q_v was the highest on the last measurement day when ratio of new root tips was higher compared to

the more mature new roots; however, Q_V did not differ between temperature treatments on day 28 and day 7 for the 10 and 20°C treatment, respectively. While Q_V on a unit root length basis was very dependent on the proportion of new root tips to more mature roots, Q_V on a whole plant basis increased with the increasing root system over the whole experimental period. In radiata pine (*Pinus radiata*) (Kaufmann 1977), soybean (*Glycine max*) and broccoli (*Brassica oleracea*) (Markhart et al 1979), new root growth was necessary to improve root hydraulic conductivity. There were significant ($p<0.01$) correlations between shoot height ($r^2=0.72$) and total leaf area ($r^2=0.92$) of *P. tremuloides* and Q_V on a whole plant basis. Plant size and leaf area were also related to root hydraulic conductivity in *Phaseolus vulgaris* (Fiscus and Markhart 1979). There was a 3.5-fold increase in Q_V when the roots grown at 5°C were tested at 5°C and then at 20°C and a 3.2-fold decrease in Q_V when roots grown at 20°C were tested at 20°C and then at 5°C. This indicates that mechanisms such as membrane permeability and/or water viscosity could also play a role in reducing the ability of the roots to deliver water to the foliage at low root temperatures (Kaufmann 1977, Wan unpublished data).

A strong relationship between Q_V and shoot development should be expected in all plants. When the Q_V is low, the flow rate of water through the plant is reduced and the stomata close to maintain a positive water balance. In turn, the closure of the stomata reduces CO_2 uptake and photosynthetic rates which results in reduced carbon production for growth. When stomatal closure is not sufficient to reduce water loss, the resulting decline in tissue water content can inhibit cell elongation and other growth processes.

At 20°C, the stabilisation of stomatal conductance (g_S) and shoot water potential (ψ_W) values by day 14, suggests that by this time there was a sufficient amount of new roots to maintain high rates of g_S in the existing leaves. The further 10-fold increase in Q_V on a whole plant basis between days 14 and 28 was probably related to the increased demand for water of the newly added foliage. By day 28 at the lower root temperatures, there were still not sufficient new roots and Q_V to reach a stable g_S or ψ_W (Figures 2-2a,b,c). In *P. ponderosa*, root temperature also had a more pronounced effect on g_S after new roots

were developed (Lopushinsky and Max 1990). It appears that, in *P. tremuloides*, high rates of Q_V are essential to maintaining high g_S and net photosynthesis of leaves. Our data suggest that at soil temperatures <10°C, new root growth, Q_V, g_S, and net photosynthesis in *P. tremuloides* would be suppressed for at least a month after air temperatures were favourable for growth.

Transferring *P. tremuloides* seedlings from 5 and 10°C to 20°C for 24 hours significantly increased Q_V and ψ_W but not g_S. It is possible this stomatal response, despite increased Q_V, is due to the low root temperature induced accumulation of ABA that inhibits stomatal opening (Chen et al 1983, Zhang et al 1987). Water potential in plants is a function of many factors including the balance between water uptake and water loss. Therefore, both g_S (Meinzer and Grantz 1990) and root hydraulic conductivity (Lopushinsky and Kaufmann 1984) can regulate ψ_W. When root temperature changed from 20°C to 5°C, both Q_V and g_S declined within 24 hours to less than 50% of the original values, whereas ψ_W remained almost unchanged (Figures 2-3b,c,d). Therefore, the rapid stomatal closure in response to low temperature was probably responsible for the short-term maintenance of ψ_W. It is possible, that the signal inducing stomatal closure was triggered by a reduction in Q_V. Stomata can sense the non-hydraulic signals from the root system and adjust their aperture accordingly, as observed in *Commelina communis* (Zhang et al 1987), *P. radiata* (Day et al 1991) and *Abies amabilis* (Teskey et al 1983). The increase in ψ_W observed in the present study when seedlings were transferred from 5 and 10°C to 20°C in the absence of an increase of g_S may have been directly caused by an increase in Q_V, perhaps partly due to decreased water viscosity in the higher temperatures.

Low root temperature reduced both root growth and the ability of existing roots to conduct water, thus suppressing water flow from the entire root system. The time of bud break and leaf flush in *P. tremuloides* is independent of soil temperatures (Landhäusser and Lieffers 1998). Therefore the expansion of leaf area despite low soil temperatures could have the following ecological implications: Low soil temperatures may limit the

ability of roots to deliver water to the foliage when water demand is increased by high air temperatures and increased vapour pressure deficits. This effect is exacerbated by the build up of insulating grasses and other organic matter on some sites which slows soil warming in the spring (Van Cleve et al 1983, Hogg and Lieffers 1991). Cold soils are rarely immediately lethal to trees (Nambiar et al 1979, Lopushinsky and Kaufmann 1984); however, low soil temperatures could be an important factor retarding growth because of water deficit stress and vessel cavitation (Auclair 1993, Kullman 1996). We suggest that low soil temperatures may contribute to early mortality of *P. tremuloides* in some boreal forest sites, particularly after bud flushes in spring and early summer when soil temperatures are still low.

ACKNOWLEDGMENTS

We thank Laura Goodchild and Justine Karst for their assistance. This study has been supported by Ainsworth Lumber Inc., Alberta Pacific Forest Industries Inc., Daishowa Marubeni International Ltd., Millar Western Industries Ltd., Slave Lake Pulp Corporation, Weyerhaeuser Canada Ltd, Natural Sciences and Engineering Research Council (NSERC), and National Centre of Excellence-Sustainable Forest Management (NCE-SFM).

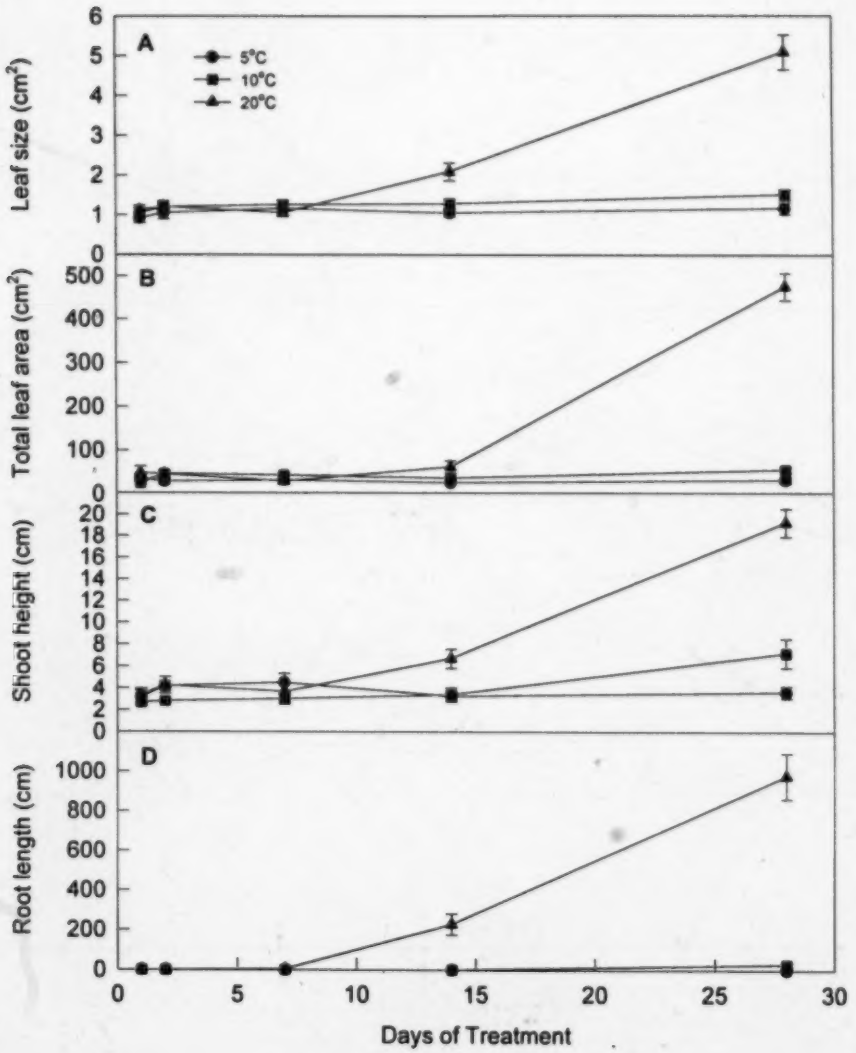

Figure 2-1. Effects of root temperature (5, 10, and 20°C) on (A) leaf size, (B) total leaf area, (C) shoot growth, and (D) root growth (± standard error (SE)) in *P. tremuloides* over a period of 28 days (n=7).

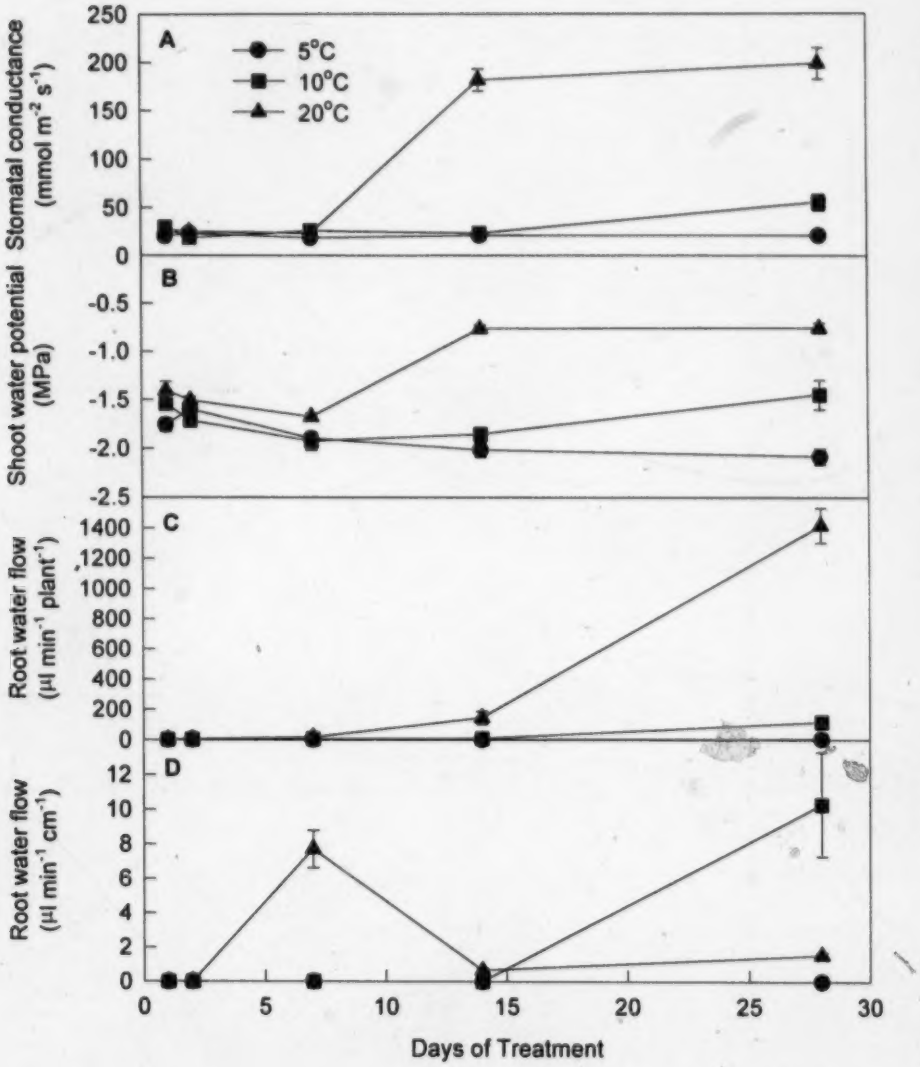

Figure 2-2. Effects of root temperature (5, 10, and 20°C) on (A) stomatal conductance, (B) shoot water potential, (C) root water flow on a whole plant basis, and (D) root water flow (± SE) on a new root unit length basis in *P. tremuloides* over a period of 28 days (n=7).

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CHAPTER THREE[1]

Effect of Low Temperature on Root Hydraulic Conductance in Aspen (*Populus tremuloides*) Seedlings

ABSTRACT

Lower temperatures significantly reduced root hydraulic conductivity and increased resistance to water flow through the roots. The increase in resistance to water flow could not be fully explained by the corresponding increase in water viscosity at low temperatures. The shapes of Arrhenius plots of root water flow and the activation energies were dependent on the direction, the sequence and the extent of temperature change. These plots suggested that the effect of low temperature on root water flow might be due to effects of low temperature on root respiration. Lowering of temperature did not induce root electrolyte leakage normally associated with cell membrane injury. Low root temperatures induced a reduction of stomatal conductance, but this reduction lagged by at least several hours behind the decline in root water flow. In contrast, when root temperatures were raised to 25°C, and root water flow presumably increased, stomatal conductance responded rapidly and was temporarily higher than that measured before the cold treatment.

Key words: Arrhenius plot, chilling, low temperature, root hydraulic conductivity, stomatal conductance.

[1] A version of this chapter has been submitted for publication. Wan X, Zwiazek JJ, Lieffers VJ, Landhäusser SM 2000. Plant, Cell and Environment.

INTRODUCTION

Low soil temperature affects growth of boreal plants throughout most of the growing season (Tryon and Chapin 1983, Hogg and Lieffers 1991). Plants that are adapted (Kaufmann 1975) or acclimated (Markhart 1979) to low growth temperatures are considered less susceptible to chilling stress compared with the plants growing under warmer climatic conditions. Low soil temperature, however, can also inhibit growth and upset water relations in boreal woody plants (Tryon and Chapin 1983, Landhäusser and Lieffers 1998). In our earlier study (Wan et al 1999), low soil temperature reduced root water flow, transpiration rates and net photosynthesis in aspen (*Populus tremuloides*) seedlings. However the mechanisms of this inhibition remain unclear. Chilling sensitivity of root water flow can be inferred by comparing root water permeability and water viscosity at different temperatures (Kaufmann 1975) and from Arrhenius plots and temperature coefficients (Q_{10}) (Markhart 1986, Hertel and Steudle 1997). Discontinuities in Arrhenius plots of root water flow have been observed at temperatures that induce injury in chilling-sensitive plants (McMurdo and Wilson 1980, Markhart 1986). The discontinuity in Arrhenius plots is interpreted as the thermotropic phase transition of membrane lipids, which might induce cell electrolyte leakage (Nishida and Murata 1996).

A reduction in root water flow at low temperatures has frequently been explained as a result of higher water viscosity (Kimmins 1987). However, low temperature could also alter root water relations by its effects on cell metabolism and membrane properties (reference by Zhang and Tyerman 1991, Kamaluddin et al 2000). In the present study, we tested the hypothesis that the inhibition of root water flow by low temperatures is due to the membrane permeability changes resulting from these metabolic changes. We examined the effects of low soil temperature on root water flow in aspen (*Populus tremuloides* Michx.) and compared root water flow resistance with water viscosity at different temperatures. We also constructed Arrhenius plots of root water flow and measured root water flow rates and stomatal conductance in response to increasing and decreasing temperatures when the temperature ranged from 4°C to 25°C. From the results

of this and previous studies (Zhang and Tyerman 1999, Wan et al 1999, Wan and Zwiazek 1999), we propose that reduced supply of root respiration products (e.g. ATP) required to open water channels may be a significant cause of root water flow inhibition in aspen.

MATERIALS AND METHODS

Experimental conditions. Aspen (*Populus tremuloides* Michx.) seedlings were grown in a growth chamber (Controlled Environments Inc. Winnipeg, MB, Canada) from seeds collected in the forest near Whitecourt, Alberta, Canada. Seeds were germinated in Petri dishes and after one day the seedlings were planted into styrofoam containers in sand and fertilized with Hoagland's mineral solution (Epstein 1972) After two months, the seedlings were gently washed free of sand with tap water and transferred to solution culture containing half-strength Hoagland's solution (Epstein 1972). The seedlings were grown an additional month in the growth chamber, set at 16-h photoperiod with 260 μmol m^{-2} s^{-1} photosynthetic photon flux density at the seedling level, 20/16°C (day/night) temperature and a constant RH of 65%. The Hoagland's solution was continuously aerated and replaced every two weeks.

Steady-state flow rates (Q_v). The rates were measured for the whole root systems using the hydrostatic pressure method (Markhart et al 1979, Rüdinger et al 1994) with some modifications (Wan and Zwiazek 1999). A glass cylinder was inserted into a pressure chamber (PMS Instruments, Corvallis, OR, USA) and filled with half-strength Hoagland's solution, which was continuously stirred with a magnetic stirrer. A detopped root system was immediately sealed in the pressure chamber. The whole root system was immersed in the solution and surrounded with a copper coil, which was connected to a circulating cooler system (HAAKE F3, Berlin, Germany) to maintain the desired root temperature (± 0.1°C). Pressure was gradually applied with compressed air and maintained during the measurements. A graduated pipette was attached with a short piece of rubber tubing to the stem protruding through the stopper in the pressure chamber. Flow

rates of whole root systems (Q_V) ($m^3\ s^{-1}$) were monitored for linearity for at least 30 min. Roots were scanned (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA), and assuming the roots were cylindrical, the root surface area was calculated by multiplying the projected area by π.

Root hydraulic conductivity (L_p). Similarly to the determination of Q_V, roots were immersed in half-strength Hoagland's solution in a pressure chamber. The solution temperature was gradually reduced and maintained at the desired temperature (20, 15, 10, or 5°C) and the pressure was increased every 40 min from zero to 0.025, 0.05, 0.075, 0.10, 0.125, 0.15, 0.2, 0.3, 0.4 and 0.5 MPa. Steady-state flow rate per unit root surface area (J_V) ($m^3\ m^{-2}\ s^{-1}$) was calculated as Q_V per unit root surface area and plotted against hydrostatic pressures. L_p ($m\ s^{-1}\ MPa^{-1}$) was calculated from the slope of the curve between 0.15 and 0.5 MPa where the relationship between pressure and J_V was linear. The reciprocal of L_p is the root resistance to water flow.

Arrhenius plots. Detopped root systems were immersed in half-strength Hoagland's solution and held in a constant pressure of 0.3 MPa while the temperature was changed between 25°C and 4°C or 25°C and 7°C in 3°C steps for Arrhenius plot determinations. The measurements started at either 25°C or at one of the low temperatures. In the latter case, the temperature was decreased from ambient to 4°C or 7°C over approximately 1 h, and after measuring Q_V, the temperature was raised in steps and then again lowered in steps to 4°C or 7°C, respectively. The temperature was monitored using a microprocessor thermometer with a fine wire type J-K-T thermocouple inserted into the pressure chamber through the rubber stopper. The chamber was pressurized and the solution was continuously stirred with a magnetic stirrer. After passing through one temperature series, the pressure chamber was opened and the solution fully aerated before continuing with the reversed temperature series. The Arrhenius plots were obtained by plotting the logarithm of Q_V against the reciprocal of the absolute temperature and the activation energy (E_a) was calculated from the slope of the whole curve of the plot for the descending temperature series.

Root temperature effects on stomatal conductance (g_s). Seedlings were transplanted to containers with aerated half-strength Hoagland's solution in a growth chamber that was maintained under the environmental conditions described above. Measurements of g_S were conducted on the sixth fully expanded leaf with a steady-state porometer (LI-1600, Li-Cor Ltd., Lincoln, NE, USA). All measurements were conducted during the light period with starting the fourth hour after the light period. An initial value of g_S was established and the temperature of the root medium was gradually reduced from 20°C to either 5°C or 10°C by a circulating cooler system (B. Braun Melsungen, Germany). After the required temperature was achieved (time 0), the measurements of g_S were repeated for up to 48 h.

In another experiment, a group of seedlings was exposed for three days to 25°C ambient day temperature (other conditions were kept the same as described for growth conditions). After three days, g_S was measured at 25°C and the root temperature was reduced to 16°C, 10°C or 4°C and maintained at these temperatures for 7 h before the measurements ending 3 h after the start of the light period. The measurements of g_S were taken twice in 30-min intervals and the root temperature was raised back to 25°C. The temperature was maintained at 25°C for up to 24 h and during that time g_S was periodically measured.

Root electrolyte leakage: To determine possible effects of low temperature on root cell membrane integrity, electrolyte leakage was measured in the roots of plants exposed to 4°C, 10°C, 16°C, and 25°C for 7 h as described by Zwiazek and Shay (1988). Roots were cut into 1-cm segments and washed with Milli-Q water. Root samples, each approximately 500 mg FW were immersed in Milli-Q water and placed under vacuum for 30 minutes. The vacuum was released and the samples incubated for 2 h at room temperature. Electrical conductivity of the solutions was measured with a conductivity meter (Mdl C33, Fisher Scientific, Nepean, ON, Canada) and then the root samples were autoclaved at 120°C for 15 minutes to measure total root electrolytes following

incubation in water for 2 h. The electrolyte leakage is expressed as percent of total electrolytes.

Root respiration. Respiration was measured as oxygen uptake using a Clark-type electrode (Yellow Springs Instrument Co., Yellow Springs, OH). Intact roots were transferred to an airtight cuvette containing aerated half strength Hoagland's solution which was continuously stirred with a magnetic stirrer. The temperature of the cuvette was controlled by a circulating cooler system (B. Braun Melsungen, Germany). Temperature were lowered from 25°C to 16°C, 10°C and 4°C. Oxygen uptake was measured every 2 min and the mean values from the second 10 min were used to calculate the respiration rates.

Reagents. All reagents were of the highest available grade and were purchased from Sigma.

Statistical analysis. The data are presented as the means of at least six replicates (seedlings). The results were analyzed by ANOVA with the Duncan's multiple comparison for root hydraulic conductivity (Fig. 3-1) and root respiration (Fig. 3-6) at different temperatures using SAS 6.12 software package (SAS Institute Inc., Cary, NC, USA). The E_a values (Fig. 3-3) and the stomatal responses to temperatures (Figs. 3-4, 3-5) were compared by two tailed *t*-test. The significance levels were all set at $\alpha = 0.05$.

RESULTS

Temperature effects on root hydraulic conductivity: Pressure-flux curves for the whole root systems at 20°C, 15°C, 10°C and 5°C showed a linear relationship between 0.15 and 0.5 MPa (Fig. 3-1). The slopes of the lines were determined over this pressure range by the linear regression analyses to calculate the L_p values. The values are $9.22 \pm 0.465 \times 10^{-8}$ m s^{-1} MPa^{-1} for 20°C, $6.78 \pm 0.547 \times 10^{-8}$ for 15°C, $4.72 \pm 0.630 \times 10^{-8}$ for 10°C and $3.12 \pm 0.518 \times 10^{-8}$ for 5°C, and the differences were ($P < 0.001$) statistically

significant. L_p at 20°C is significantly higher than that at 15°C, and the latter significantly higher than those at 10°C and 5°C. There is no significant difference in L_p between at 10°C and 5°C. Thus the relative decrease in L_p or increase in resistance to flow was very steep with declining temperatures (Fig 3-2), relative to the theoretical increase in resistance to flow that is related to the increase in water viscosity alone (Reynolds and Richards 1996).

Temperature effects on root water flow: The shapes of the Arrhenius plots and the E_a values were dependent on the direction, sequence and extent of temperature change (Fig. 3-3). In Figure 3-3a, root temperature was first reduced from 25°C to 4°C and increased back to 25°C. In Figure 3-3b, root temperature was first raised from 4°C to 25°C and then reduced back to the initial temperature. The descending temperature sequence in each graph was used to determine the activation energy E_a. The E_a value (9.15 ± 0.76 kcal mol^{-1}) starting at 25°C and moving to 4°C (Fig. 3-3a) was significantly ($p = 0.03$) higher than the E_a calculated from the descending sequence (7.50 ± 0.50 kcal mol^{-1}) where roots started at 4°C, were raised to 25°C and then descended back to 4°C (Fig. 3-3b). There were no significant differences in E_a between the roots subjected to a temperature decrease from 25°C to 4°C compared with the decrease from 25°C to 7°C (Fig. 3-3c) (9.15 ± 0.76 kcal mol^{-1} and 9.16 ± 0.87 kcal mol^{-1}, respectively). When the temperature descended first and then ascended, the points from the ascending temperatures were hysteretic to those from the descending temperature, and the hysteresis from 4°C to 25°C was greater than that from 7°C to 25°C.

Root temperature effects on stomatal conductance: When the root temperature was decreased from 20°C and held at 5°C and 10°C, g_s fluctuated and decreased to about 40% of the control value after 12 h for 5°C and after 35 h for 10°C (Fig. 3-4). At 5°C root temperature, the plants lost turgor and the leaves wilted within 24 hours.

When the root temperature was reduced from 25°C to 16°C or 10°C for 7 h, g_s values did not show a significant decline (Fig. 3-5). When the temperature returned to 25°C, g_s

increased for 2 h above the initial level measured at this temperature ($p < 0.01$) and then returned to the initial value. When the root temperature decreased from 25°C to 4°C for 7 h, g_S significantly declined ($p < 0.01$) (Fig. 3-5). The value of g_S also increased when the temperature returned to 25°C, however, it did not reach the initial, pre-decrease level ($p = 0.013$) until 22 h later (Fig. 3-5).

Root respiration: There was significant difference ($p < 0.001$) in root respiration when the roots were subjected to the different temperatures (Fig. 3-6). Low root temperature inhibited the respiration.

Root electrolyte leakage: Electrolyte leakage was similar in roots exposed to 25°C, 16°C, 10°C and 4°C for 7 h and measured respectively 19.1%, 20.0%, 19.05 and 18.5% of total electrolytes after 2-h incubation time.

DISCUSSION

Kaufmann (1975) noted that chilling-sensitive trees have greater water transport resistance at 8°C soil temperature compared with the chilling tolerant trees. This difference in water transport sensitivity to chilling stress suggests that the reduction in root water flow at low temperature may not be a simple consequence of increased water viscosity at low temperatures as suggested by (Kaufmann 1975, 1977). In the present study, a decline in water viscosity could only partly account for decreased root water flow in aspen at low root temperature (Fig. 3-2). At low soil temperature, water uptake is a limiting factor affecting transpiration rates and plant water status (Wan et al 1999, Wan and Zwiazek 1999). The effect of low temperature on root water flow is relatively rapid (Bolger et al 1992, Bigot and Boucaud 1994, Fennell and Markhart 1998).

In our study, water flow rates stabilized about 20 minutes after the root temperature was reduced. Lowering the temperature from 20°C to 10°C for 20 minutes triggered a reduction in root water flow by about 50% (Fig. 3-3). However, it took 30 h following the

temperature decline from 20°C to 10°C before g_s showed a significant decline (Fig. 3-4). There are two possible explanations for the delay in g_s response to root chilling. As part of the drought resistance strategy, roots can usually supply more water than required to replenish transpirational losses (Kramer 1983). The leaves received initially sufficient amount of water under the low temperature conditions. In addition, water stored in the stem can temporarily buffer the shortage of water supply (Schulze et al 1985, Goldstein et al 1998). However, this relatively slow stomatal response could be also explained by osmotic adjustment of the guard cells and resulting turgor maintenance during the initial stages of reduced water supply.

In an earlier study (Wan and Zwiazek 1999), we found that mercury inhibition of root water transport in aspen was temperature-sensitive suggesting that the functioning of water channels is temperature-dependent. It is interesting that the low temperature reduction of water flow rate was not immediately reversible by higher temperatures. Electrolyte leakage was similar from roots exposed to temperatures ranging from 4°C to 25°C indicating that the low temperatures studied did not cause membrane injury. It is possible that low temperature induced conformational changes of the water channel proteins and affected their gating function (Hladky and Haydon 1972, Tyerman et al 1999). Functioning of water channels is linked to respiration (Zhang and Tyerman 1999, Kamaluddin et al 2000), and their opening mechanism may involve phosphorylation (Maurel 1997), therefore root respiration inhibition at low temperature (Fig. 3-6) may be indirectly responsible for the decline in root water flow. It is also evident that respiration inhibition by sodium azide caused a rapid decline in root water flow in aspen seedlings (our unpublished data).

In the present study, temperature decline from 25°C to 4°C had a greater effect on plot lags and on the activation energies when the temperatures increased back step by step compared with the decrease from 25°C to 7°C. When the measurements started at 4°C and the temperature was raised to 25°C and then lowered back to 4°C, water flow rates at lower temperatures were higher on the descending sequence than those on the ascending

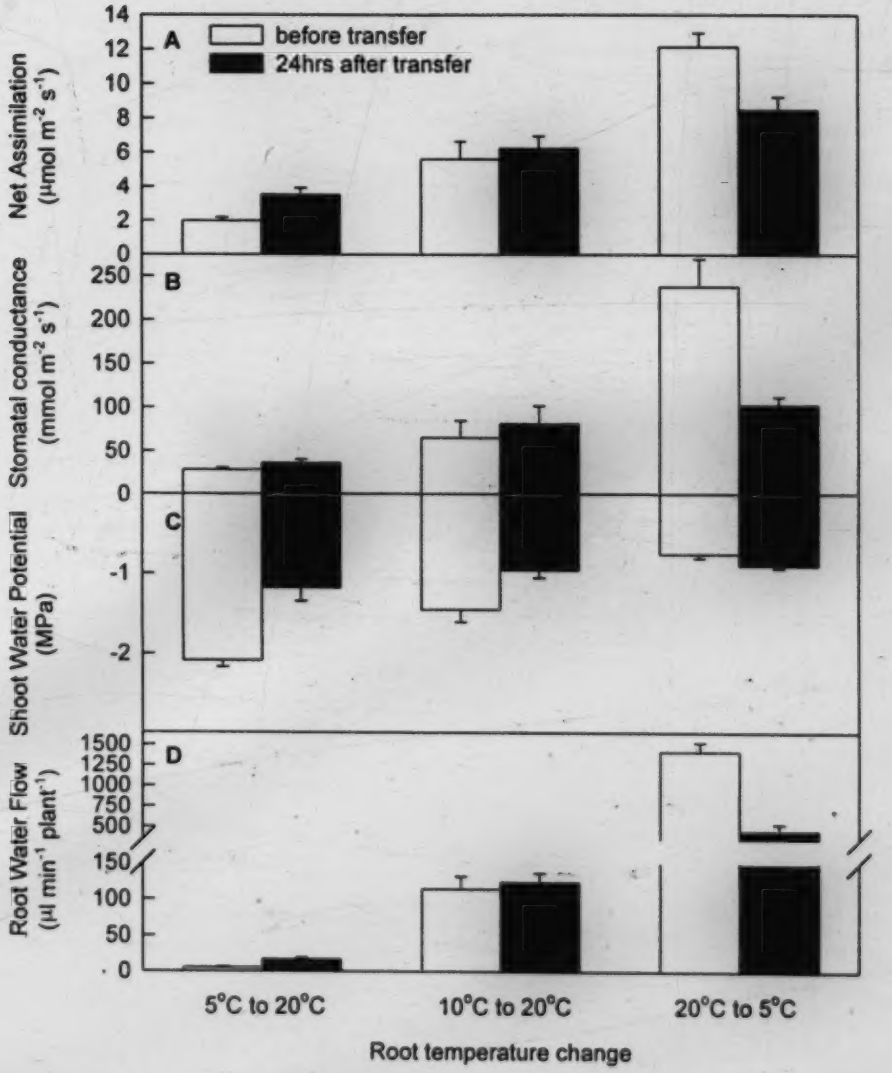

Figure 2-3. Response of (A) net assimilation, (B) stomatal conductance, (C) shoot water potential, and (D) root water flow (± SE) in *P. tremuloides* 24 hours after transferring seedlings grown at 5 and 10°C to 20°C and seedlings grown at 20°C to 5°C root temperature (n=7, see M&M part II for details).

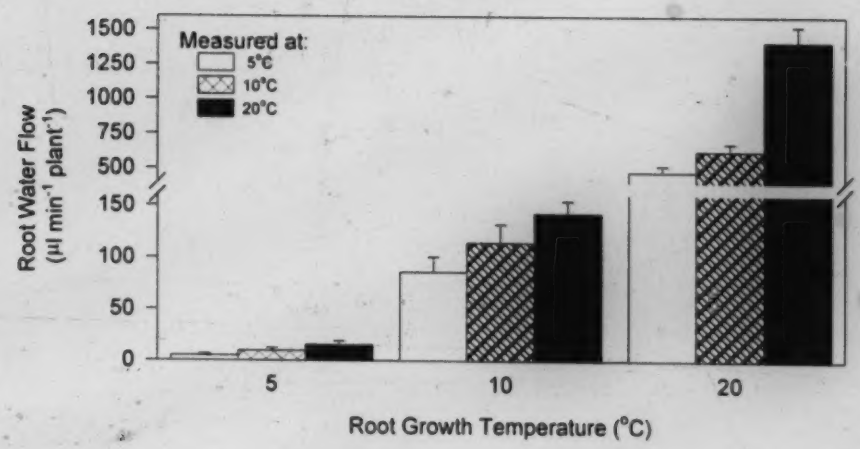

Figure 2-4. The short-term response of root water flow (± SE) in *P. tremuloides* root systems which had been growing for 28 days at their respective root temperatures were then measured at 5, 10, and 20°C (n=7).

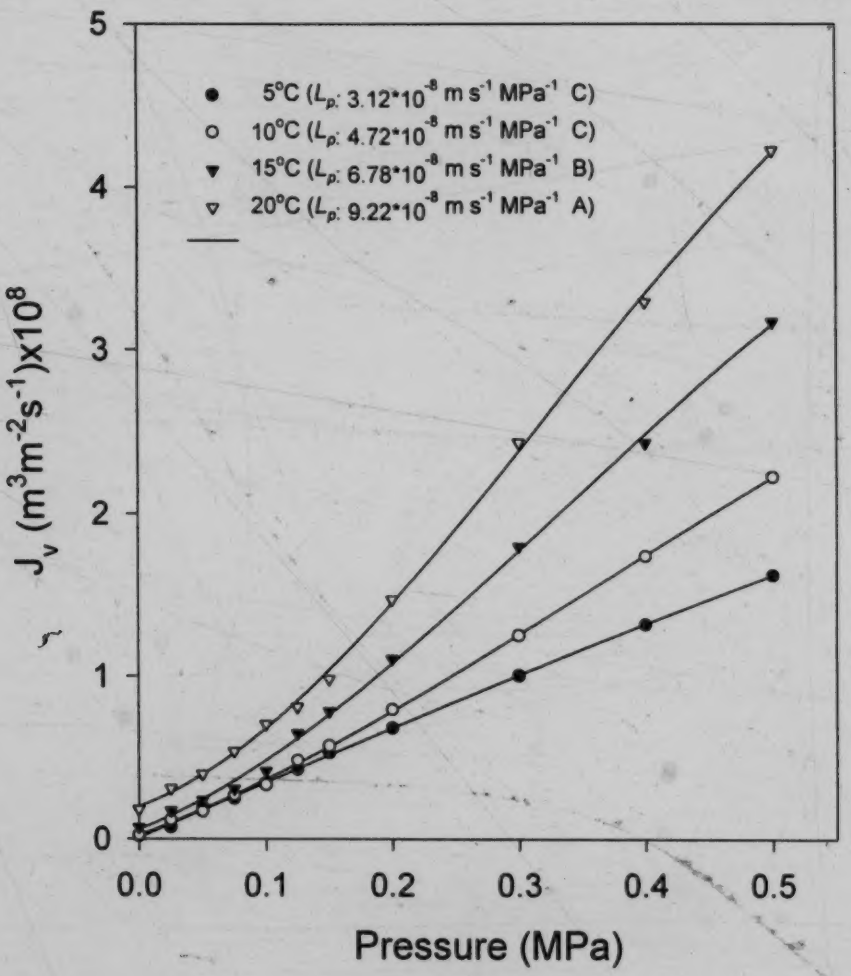

Figure 3-1. Pressure-flow relationship in aspen root systems at different temperatures. Means are shown (n = 6).

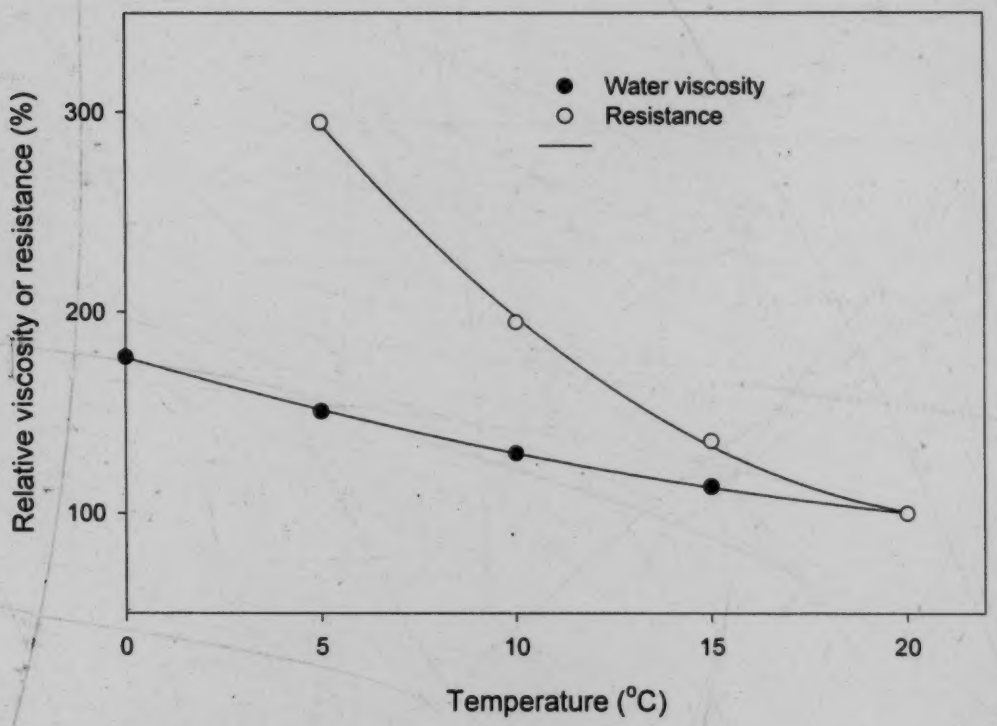

Figure 3-2. Relative viscosity of water and relative resistance (the reciprocal of root hydraulic conductivity calculated from plots in figure 3-1) for water flow through aspen roots at low soil temperature.

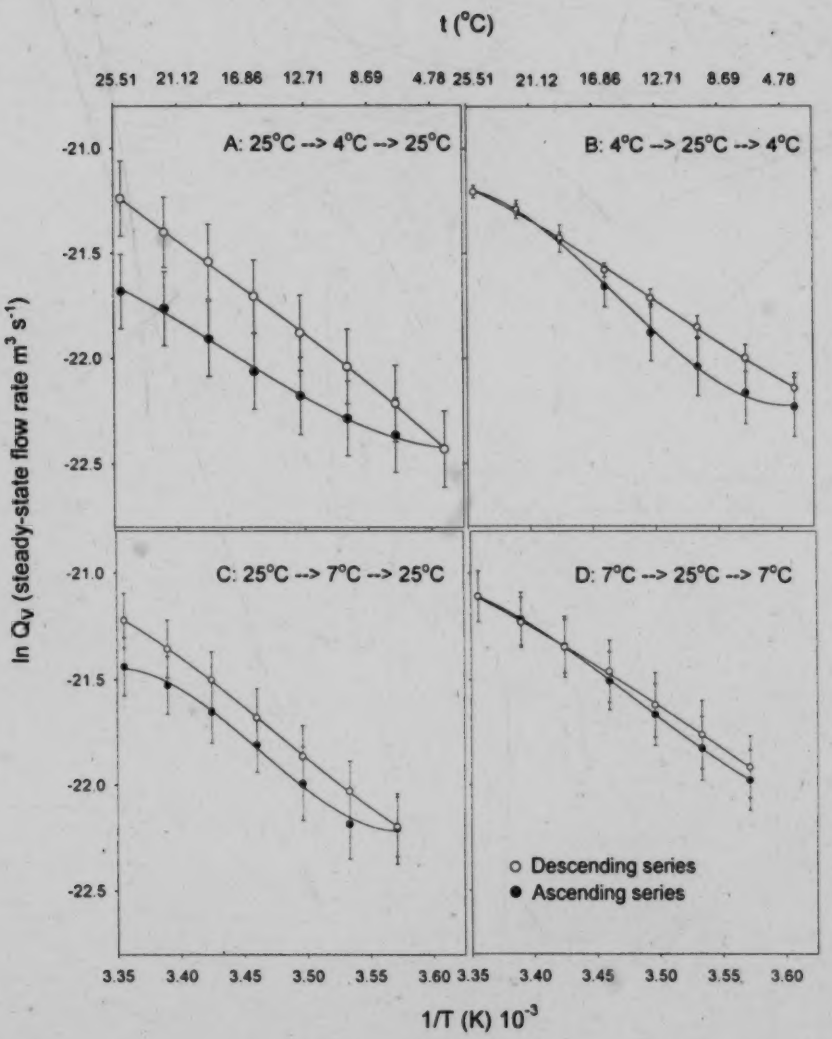

Figure 3-3. Temperature effects on water flow through aspen roots at constant hydrostatic pressure of 0.3 MPa. Steady state water flow was continually measured in (A and C) temperatures descending to 4°C or 7°C followed by ascending temperatures to 25°C. (B and D) temperatures ascending series followed by decreasing temperature series. Means are shown (n = 6).

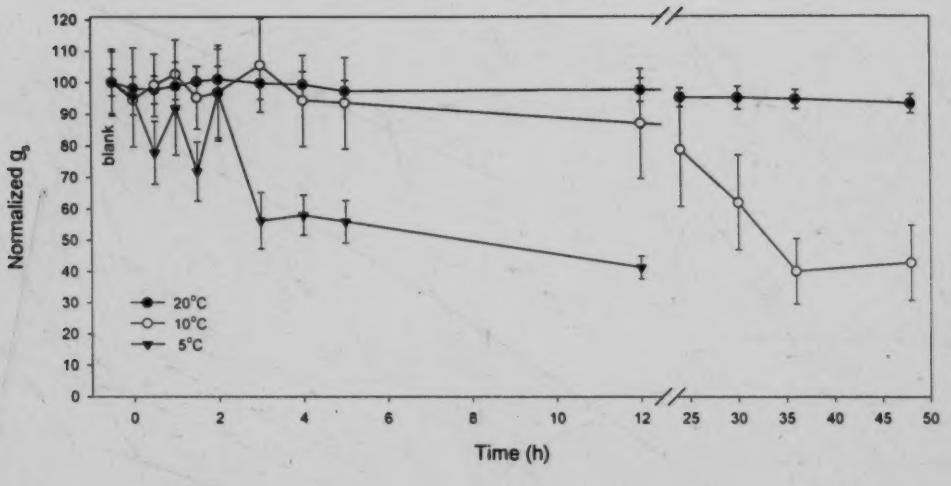

Figure 3-4. Effects of root temperature on stomatal conductance (g_S) in aspen seedlings. The values for g_S were normalized to those before temperatures changed (20°C). Light regime is kept as the same diurnal rhythm as the growth condition of the seedlings. Night periods started after twelfth and thirty-sixth hour. Means ± SE (n = 6) are shown.

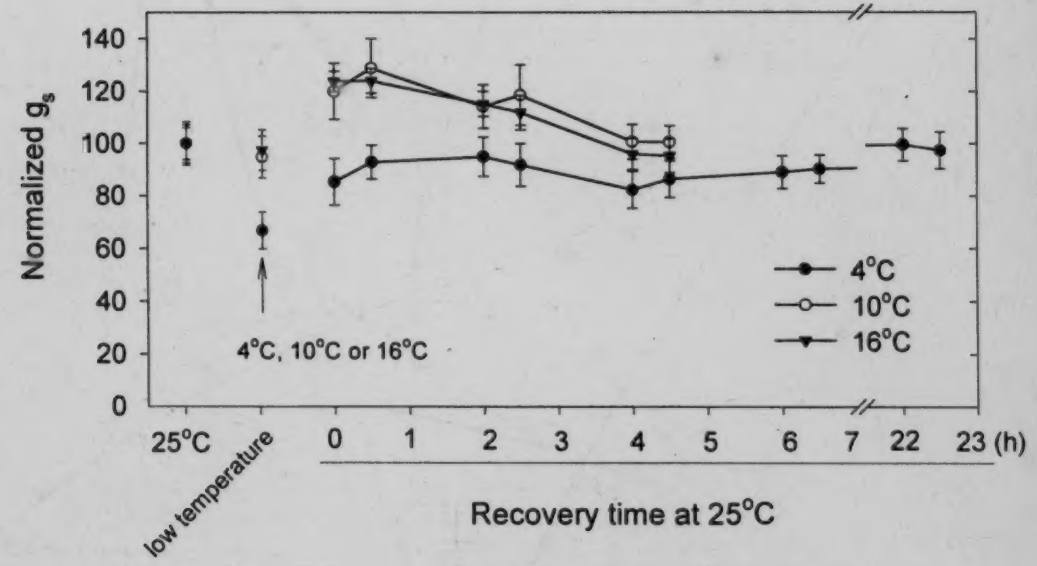

Figure 3-5. Response of stomatal conductance to root temperature change. Plants were held at 4, 10 or 16°C for 7 hours, indicated by the low temperature position on the graph. Roots were then warmed and g_s was measured at 25°C (time 0 and later). The values for g_S were normalized to those before temperatures changed (25°C). Means ± SE (n = 6) are shown.

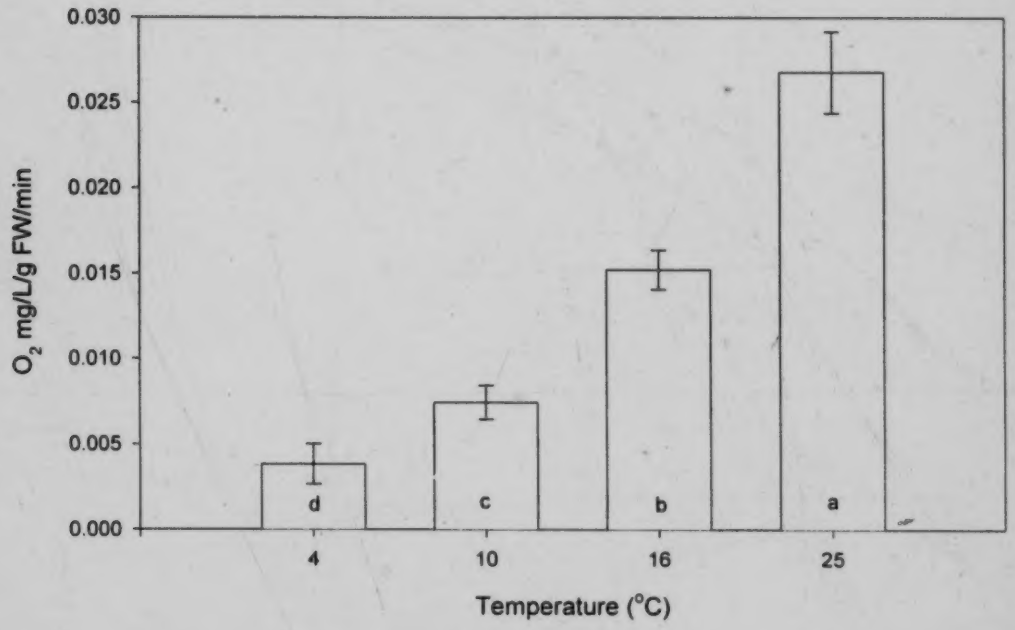

Figure 3-6. Effect of temperature on root respiration. Bars with the different letters are significantly different at the 0.05 level. Means ± SE (n = 6) are shown.

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CHAPTER FOUR[1]

Mercuric chloride effects on root water transport in aspen seedlings

ABSTRACT

Mercuric chloride (0.1 mM) reduced pressure-induced water flux and root hydraulic conductivity in the roots of one-year-old aspen (*Populus tremuloides* Michx.) seedlings by about 50%. The inhibition was reversed with 50 mM mercaptoethanol. Mercurial treatment reduced the activation energy of water transport in the roots from 10.82 ± 0.700 kcal mol^{-1} to 6.67 ± 0.193 kcal mol^{-1} when measured over the 4°C - 25°C temperature range. An increase in Rhodamine B concentration in the xylem sap of mercury-treated roots suggested a decrease in the symplastic transport of water. However, the apoplastic pathway in both control and mercury-treated roots constituted only a small fraction of the total root water transport. Electrical conductivity and osmotic potentials of the expressed xylem sap suggested that 0.1 mM $HgCl_2$ and temperature changes over the 4°C - 25°C range did not induce cell membrane leakage. The 0.1 mM $HgCl_2$ solution applied as a root drench severely reduced stomatal conductance in intact plants and this reduction was partly reversed by 50 mM mercaptoethanol. In excised shoots, 0.1 mM $HgCl_2$ did not affect stomatal conductance suggesting that the signal which triggered stomatal closure originated in the roots. We suggest that mercury-sensitive processes in aspen roots play a significant role in regulating plant water balance by their effects on root hydraulic conductivity.

[1] A version of this chapter has been published. Wan X, Zwiazek JJ 1999. Plant Physiology 121, 939-946.

INTRODUCTION

Several criteria have been used to infer the presence of water transporting channels in cell membranes. These include a high ratio of osmotic to diffusional water permeability ($P_f/P_d > 1$), low Arrhenius activation energy ($E_a < 6$ kcal·mol^{-1}) for water transport, and its reversible inhibition by mercury sulfhydryl reagents (for reviews, see Chrispeels and Agre 1994, Verkman et al 1996, Maurel et al 1997). Diffusional transport of water is through the lipid bilayer. The lipid bilayer packs more tightly at lower temperature, making the transport of water highly temperature-dependent (Chrispeels and Agre 1994). Therefore, in the diffusional transport, E_a is usually high, above 10 kcal mol^{-1} (Macey 1984). Osmotic water transport is via water channel proteins (aquaporins), which have been found in the tonoplasts (Maurel et al 1993) and plasma membranes (Kammerloher et al 1994) of plants. The presence of pores facilitates bulk water flow across the membrane. It is generally acknowledged that the osmotic transport of water possesses less temperature dependence, and lower E_a (< 6 kcal mol^{-1}) than the diffusional transport (Finkelstein 1987, Chrispeels and Agre 1994). The osmotic transport is characteristically inhibited by mercurial reagents, which react with sulfhydryl groups in channel proteins and result in closure of the water channels. This closure inhibits osmotic transport and increases E_a to the level of diffusional permeability (Macey 1984). An inhibition of water transport by mercury was reported in cell membranes isolated from higher plants (Niemietz and Tyerman 1997, Maurel et al 1997) and in whole root systems (Maggio and Joly 1995, Carvajal et al 1996). However the effects of mercury reagents on E_a have not been investigated in higher plants.

Based on the composite transport model (Steudle and Frensch 1996), water transport is via three parallel pathways, that is, apoplastic, symplastic and transcellular. Both symplastic and transcellular pathways are often referred to as the cell-to-cell pathway (Steudle and Frensch 1996). In the present study, we use the term symplastic transport to describe the cell-to-cell transport of water involving both the transmembrane transport and that through the plasmodesmata. Due to the cell wall continuum in whole plants,

possible effects of $HgCl_2$ on cell walls must be considered. We studied these effects with a fluorescent dye (rhodamine B), which is transported only through the apoplast (Skinner and Radin 1994).

The importance of root regulation of water flow in plant water relations has received relatively little attention. In the present study, we employed a pressure-flux approach (Markhart et al 1979, Rüdinger et al 1994) to examine the effects of $HgCl_2$ on the properties of water transport and its E_a in the intact root systems of aspen (*Populus tremuloides* Michx.) seedlings grown in solution culture. We also studied the impact of mercury-sensitive root water transport on stomatal conductance. Since mercury chloride may also act as a general metabolic inhibitor, we investigated its effect on root oxygen uptake. Based on the results of our study, we suggest that the mercury-sensitive processes of water transport in aspen roots affect plant water balance by regulating root hydraulic conductivity which, in turn, triggers changes in stomatal opening.

MATERIALS AND METHODS

One-year-old aspen (*Populus tremuloides* Michx.) seedlings were grown in the greenhouse from seed collected at Drayton Valley, Alberta, Canada. The plants were grown in plastic containers containing garden soil and were set dormant before being transferred to solution culture. The roots of dormant seedlings were gently washed free of soil with the tap cold water and the seedlings were transferred to solution culture containing half-strength modified Hoagland's solution (Epstein 1972). The plants were grown for further 1.5 months in a growth chamber (Controlled Environments Inc. Winnipeg, MB, Canada). The growth chamber was set at the 16-h photoperiod with 260 μ mol m^{-2} s^{-1} PPFD at the seedling level, 22/18°C (day/night) temperature and a constant RH of approximately 65%. The Hoagland's solution was continuously aerated and replaced every two weeks.

sequence. This could indicate that root energy reserves were quickly replenished at higher temperatures and that some were still available to water channels when the temperature was lowered. Respiration energy requirements for the opening of water channels could explain rapid loss of water balance in plants treated with metabolic inhibitors.

The slow recovery of stomatal conductance in plants with the roots exposed to 4°C and then transferred to 25°C indicates that the inhibition of root water flow was not immediately reversible. It is noteworthy that stomatal conductance responded differently to an increase compared with a decrease in root water flow. As discussed above, there was a relatively long delay in stomatal response triggered by declining temperatures (Fig. 3-4) and a relatively fast response to increasing temperatures (Fig. 3-5). Interestingly, the initial stomatal response to 25°C in plants with the roots exposed to 10°C and 16°C was an increase of the stomatal conductance above the initial level measured at 25°C (Fig. 3-5). These results imply that under the study conditions root water flow at 25°C could supply more water to the guard cells than necessary to maintain the opening of the stomata but despite that the stomata were not open to their full capacity. There seems to exist a threshold response of stomatal regulation. When deprivation of water does not reach a threshold the guard cells down adjust their osmotic potential, by which they obtain water from the surrounding ordinary epidermis cells to keep the stomatal aperture, in favour of gas exchange. If the deprivation of water is severe enough over the threshold, the guard cells reduce the solute content and deflate, and the stomata close to prevent excessive water loss. In the former case, increasing water supply, by rising root temperature, immediately swells the guard cells and widens the stomatal aperture because the guard cells possess lower water potential and the surrounding epidermis cells have not built up their turgor. Later on, when the ordinary epidermis cells also inflate, the stomatal aperture returns to the original magnitude. Stomatal aperture depends on both the guard cells and the surrounding epidermis cells turgor (Lambers et al 1998). There is another scenario in the latter case, in which some metabolic processes (e.g. abscisic acid accumulation) have been involved in initiating stomatal closure and preventing opening (Raschke 1987). Therefore, re-supplying leaves with water can not immediately restore

the stomatal opening to the original level.

In conclusion, our study showed that the decline in root water flow rates under low temperature conditions could not be fully explained by an increase in water viscosity. The response of water flow to declining and increasing temperatures reported in this study and reported earlier (Wan et al 1999, Wan and Zwiazek 1999) indicates that water channels are involved in this response. We suggest that respiration play an important role in regulating the permeability of water channels.

ACKNOWLEDGMENTS

This study was funded by research grants from the Natural Sciences and Engineering Research Council of Canada and Sustainable Forest Management Network of Centres of Excellence.

The exudates were collected when the measurement temperature was at 25°C. For control group, there were two 25°C points, one at the beginning of the descending temperature series and the other at the end of the ascending temperature series. They are referred to as descending sap and ascending sap, respectively. For the $HgCl_2$-treated group, before $HgCl_2$ addition, a Q_v value was measured at 25°C as a blank reference. Thereafter, $HgCl_2$ was added to the root medium and the temperature was changed as for the control group, i.e. from 25°C to 4°C and back to 25°C. Therefore, there are three 25°C points in the treatment group and are referred to as the reference sap, descending sap and ascending sap, respectively. The xylem saps were collected for osmotic potential and electrical conductivity determinations. Osmotic potentials were measured with a thermocouple psychrometer (HR-33T, 5112, Wescor, Inc., Logan, UT, USA) and a C52 sensor, in a dew point mode, and the electrical conductivities were determined with a conductivity meter (Mdl C33, Fisher Scientific, Nepean, ON, Canada).

Determinations of L_p. Roots were immersed for 30 min in half-strength Hoagland's solution in a pressure chamber at 22°C. Water or $HgCl_2$ was added as previously described, and the pressure increased every 30 min from 0 to 0.025, 0.05, 0.075, 0.10, 0.125, 0.15, 0.2, 0.3, 0.4 and 0.5 MPa. J_v was calculated as Q_v per unit root surface area and plotted against hydrostatic pressures. L_p was calculated from the slope of the curve between 0.15 and 0.5 MPa where the relationship between pressure and J_v was linear, and it is expressed in m s^{-1} MPa^{-1}.

Symplastic and apoplastic pathways. Rhodamine B (RB) was used to trace root water transport and to detect the effect of mercuric chloride on the symplastic and apoplastic flux. Rhodamine B is a fluorescent dye believed to be transported only through the apoplast (Skinner and Radin 1994). A root system was sealed in a pressure chamber filled with a half-strength Hoagland's solution and the chamber pressurized to 0.3 MPa. The Q_v value was measured and RB was added to a final concentration of 20 μg mL^{-1}. The Q_v value was measured again for 1 h and $HgCl_2$ was added for another 1 h and Q_v measured

again over the 1-h incubation period. The first 30-min xylem exudates were discarded and the rest collected to measure RB concentration, electrical conductivity and osmotic potential. The concentration of RB was measured using a Sequoia-Turner model 450 fluorometer (Apple Scientific Inc., Chesterland, OH, USA). The excitation and emission wavelengths were 520 and 605 nm, respectively. A standard curve of known RB concentrations was established to calculate RB in xylem exudates. The apoplastic flow was estimated by dividing tracer concentration in the expressed xylem exudate by its concentration in the root incubation solution.

Measurements of g_s. For g_S measurements in intact plants, the seedlings were grown in aerated half-strength Hoagland's solution in a growth chamber that was maintained under identical environmental conditions as those described earlier. Measurements of g_S were conducted on the sixth fully expanded leaf from top with a steady-state porometer (LI-1600, Li-Cor Ltd., Lincoln, NE, USA). In control (untreated) group, g_S was measured in 30-min intervals for 4 h and after 16 h. In treated plants, g_S was measured before and after $HgCl_2$ was added to the incubation solution to a final concentration of 0.1 mM. The measurements were conducted in 30-min intervals for 3 h. Subsequently, ME was added to a concentration of 50 mM and g_S was measured after 30 min, 1 h and 12 h. All measurements were conducted during the light period.

In the second experiment, excised shoots were used instead of intact seedlings. The seedlings were placed in a half-strength Hoagland's solution in the dark growth chamber for 4 h and the shoots were excised at the root collar under the solution. Excised shoots were immersed in half-strength Hoagland's solution and exposed to light in the growth chamber. The measurements of g_S were conducted in 1-h intervals for the first 4. For $HgCl_2$ treatment, the shoots were placed in half-strength Hoagland's solution containing 0.1 mM $HgCl_2$ and g_S was measured at the same times as in the controls.

Reagents. All reagents were of the highest available grade and were purchased from Sigma.

Statistical analysis. The data are presented as the means of at least four replicates (seedlings). The data of root respiration were analyzed using one-way ANOVA and with Duncan multiple comparison by SAS 6.12 software package (SAS Institute Inc., Cary, NC, USA). Two tailed *t*-test was performed for all comparisons between control and $HgCl_2$-treated data. All statistically significant differences were tested at the $p \leq 0.05$ level.

RESULTS

The concentrations of $HgCl_2$ ranging from 0.05 mM to 0.25 mM resulted in a similar level of inhibition of Q_V within 60 min. from application (Fig. 4-1). The highest concentration (0.5 mM) inhibited Q_V more rapidly than the lower concentrations and the lowest concentration (0.025 mM) acted relatively slowly on Q_V and was less effective than the higher concentration treatments (Fig. 4-1).

When the roots were held at the constant pressure of 0.3 MPa, $HgCl_2$ caused a rapid decrease in J_V (Fig. 4-2). Within ten minutes following injection of ME into the solution, this inhibition of J_V. was almost completely reversed (Fig. 4-2). The results calculated from 8 replicates indicated that $HgCl_2$ inhibited J_V by 47% (± 3.17%) and that J_V returned to 91% (± 3.36%) of the original values after adding ME. There was no significant difference in J_V of the control roots over the 2-h measurement period.

Pressure-flux curves from $HgCl_2$-treated and control roots (n = 6) showed a linear relationship between 0.15 and 0.5 MPa (Fig. 4-3). The L_p values calculated over this range were respectively $9.71 \pm 0.836 \times 10^{-8}$ m s^{-1} MPa^{-1} and $4.88 \pm 0.263 \times 10^{-8}$ m s^{-1} MPa^{-1} for the control and $HgCl_2$-treated roots and the difference was statistically significant.

Both control and treated roots had linear Arrhenius plots for Q_V (Fig. 4-4). The treatment with $HgCl_2$ reduced not only Q_V but also E_a. The E_a value was 10.82 ± 0.7 and 6.67 ±

0.193 kcal mol^{-1}, respectively, for the control and treated roots and the difference was highly significant. In control, but not $HgCl_2$-treated roots the Arrhenius plots were not linear in higher temperatures when measured for ascending temperatures following temperature decrease to 4°C (Fig. 4-5). Neither $HgCl_2$ nor temperature changed osmotic potentials of the xylem exudates (Tab. 4-I). However, $HgCl_2$ treatment increased the electrical conductivity of the expressed sap (Tab. 4-I). In control roots, the electrical conductivity increased after the temperature descended to 4°C and then ascended back to 25°C.

RB concentration in the xylem sap of the control roots was about 0.01% of that in the incubation solution. In $HgCl_2$-treated roots, the decrease in Q_V was accompanied by an increase in the concentration of RB and in the electrical conductivity of the expressed xylem sap (Tab. 4-II). However, there was no difference in osmotic potentials of the control and $HgCl_2$-treated exudates (Tab. 4-II).

$HgCl_2$ significantly inhibited stomatal conductance in intact seedlings. After three hours of incubation in $HgCl_2$, the g_S rates declined from about 23 mmol m^{-2} s^{-1} to less than 7 mmol m^{-2} s^{-1} (Fig. 4-6A). The inhibition of g_S was only partly reversed by 50 mM ME. After one hour, ME resulted in a significant ($p = 0.013$) increase in g_S to above 10 mmol m^{-2} s^{-1} (Fig. 4-6A). Over the experimental period, no significant changes in g_S were detected in control seedlings ($p = 0.318$).

In excised shoots, g_S rates remained stable in the first 12 hours and thereafter declined with time in both control and $HgCl_2$-treated plants. However, there was no significant difference in g_S between the control and treated shoots (Fig. 4-6B).

Treatment with 0.1 mM $HgCl_2$ did not significantly reduce root respiration in the first hour (Fig. 4-7). However after 4 h of treatment, 0.1 mM $HgCl_2$ caused a reduction in oxygen uptake by 17% while that in 0.5 mM $HgCl_2$ was reduced by 30-43% (Fig. 4-7).

DISCUSSION

Mercury reversibly inhibits the bulk water transport across membranes in animal (Pratz et al 1986, Meyer and Verkman 1987) and plant cells (Maggio and Joly 1995, Carvajal et al 1996, Niemietz and Tyerman 1997, Maurel et al 1997). This reversible inhibition is used to demonstrate the existence of proteinaceous water channels (Chrispeels and Maurel 1994). Our experiment followed the methodology used by Maggio and Joly (1995), employing the whole root system and the pressure-flux approach. The result of the kinetics of reversible mercurial inhibition of water flow suggested the presence of water channels in aspen roots. $HgCl_2$ inhibited root water flow in aspen by decreasing root hydraulic conductivity. This suggests that root water channels play an important role in regulating plant water relations. The pressure-flux curves, in untreated controls and $HgCl_2$-treated roots were consistent with the theory (Fiscus 1975) and observations (Lopushinsky 1964, Markhart et al 1979, Jackson et al 1996). The relationship between J_v and applied pressure was highly linear in pressures above 0.15 MPa. Below this point, the curve was not linear and did not cross at zero J_v, especially for controls, in which water flows were observed at 0 MPa of pressure due to root pressure described by Fiscus (1975). The values of J_v and L_p observed in this experiment were lower compared with those in tomato (Maggio and Joly 1995), soybean (Fiscus 1977), bean (Fiscus 1981), and maize (Zhu and Steudle 1991). This is in agreement with earlier observations that the roots of woody plants have lower permeability to water compared with herbaceous species (Steudle and Meshcheryakov 1996).

A low Arrhenius activation energy (E_a< 6 kcal·mol^{-1}) for water transport is among the typical features of the membranes with water-transporting pores (Chrispeels and Agre 1994, Verkman et al 1996, Niemietz and Tyerman 1997, Maurel et al 1997), while transport through the membrane lipid bilayer is associated with high Arrhenius E_a values. Mercurials can increase the activation energy of water permeation facilitated by water channels (Macey 1984, Meyer and Verkman 1987, van Hoek et al 1990). However, in our study $HgCl_2$ significantly reduced E_a values for Q_v in roots and more studies will be

required for a proper explanation of these results. The apoplastic pathway in the intact roots may be different from that in the isolated membrane vesicles or cells. The proportion of apoplastic flow increased in the $HgCl_2$-treated roots. However, this increase may not necessarily be the reason for E_a reduction. We assumed that the mercuric inhibition of Q_v was due to blocking of the water channels. It is commonly accepted that water channels are not temperature-sensitive. The Q_{10} value for water transport through an aqueous pore is essentially the same as that for the viscosity of water, which is about 1.25 (Finkelstein 1987). From this point of view, the apoplast is similar to the water channels. Therefore the inhibition of the temperature-insensitive processes should not be expected to reduce temperature sensitivity for the whole water transport. At the present time, we cannot conclude with certainty that the water channels in aspen are sensitive to temperature. Nevertheless, the results suggest that the mercury-sensitive processes are also temperature-sensitive. If the effect of mercury chloride is mainly on water channels as reported for the individuals cells and isolated membrane vesicles (Macey 1984, Meyer and Verkman 1987, Pratz et al 1986, Niemietz and Tyerman 1997, Maurel et al 1997), the channels may indeed be temperature-sensitive. However, we cannot exclude the possibility that other, temperature-sensitive processes involved in root water transport are affected by mercury resulting in this effect. The increased sensitivity of root water flow to low temperatures that we found in aspen could be an adaptive feature if present in other perennial plants that are exposed to seasonal low temperatures. This increased sensitivity could allow the plants to regulate root water flow at the low temperatures to prevent xylem cavitation at the end of the growth season and prepare for winter rest.

It is often assumed that the water-transporting pores are rigid and hardly change their shape or size with changing temperature while the water permeability of the phospholipid bilayer is temperature-dependent (Chrispeels and Agre 1994). However, protein pores do not have to be rigid. E_a will depend on the nature of the rate-limiting barrier for water movement and on the energetics of the water-pore interactions (Verkman et al 1996). Moreover, Arrhenius plots of water movement in soybean (Markhart et al 1979) and in renal proximal tubule cell membranes (Meyer and Verkman 1987) were found to be non-

linear. In our experiment, when the temperature decreased from 25°C to 4°C and then ascended back to 25°C, control roots did not yield a linear Arrhenius plot (Fig. 4-5). This did not appear to be due to membrane damage by the low temperatures. The ascending sap had a higher electrical conductivity than the descending one (Tab. 4-I), however, the increased conductivity was likely due to the relative increase in the apoplastic transport after the symplastic transport was inhibited by the low temperatures. Unlike in control seedlings, the $HgCl_2$-treated roots had fully reversible linear plots (Fig. 4-5). This suggests that following mercuric treatment, the roots lost their sensitivity to low temperature. It is possible that the *in vitro* conditions, used in many previous studies, may affect differently the function of water transport than those in the intact roots.

In our experiment, the concentration of RB in the xylem sap expressed from the control roots was only 0.012% of that in the incubation solution, suggesting that only a very small fraction of water was transported in the roots through the apoplast. The concentration of RB in the xylem sap of $HgCl_2$-treated roots increased to 0.025% of that in the solution. This indicates some increase in the apoplastic water transport, but also suggests the shift from bulk to diffusional water transport across the membranes, since the concentration of RB was only a small fraction of that present in the incubation solution. Fluorescent tracer results must be, however, interpreted with caution for water movement. The molecule weight of RB is 479 D, higher than that of water molecule. The rates of transport of fluorescent tracers and water through the apoplast may be different due to their different molecular sizes (Yeo et al 1987, Hanson et al 1985). Therefore, the concentration of RB in the xylem sap gives an indication rather than a precise estimate of the symplastic to apoplastic ratio of water transport.

Electrical conductivity increased along with an increase in RB concentration in the xylem sap of $HgCl_2$-treated roots and in those exposed to low temperatures (Tabs. 4-I and 4-II). These results confirm the increase in the apoplastic transport of the treated roots as the apoplastic transport does not selectively filter out ions present in the root media (Peterson et al 1981, Yeo et al 1987). It is interesting that the increase in the electrical conductivity

of the xylem sap was not reflected by a decrease in osmotic potentials. This suggests a change in the solute composition of the xylem sap that resulted from the change in the water transport pathway.

$HgCl_2$-treated plants with intact roots closed the stomata and showed signs of wilting within 2-3 hours following treatment. The stomatal closure was partly reversed with 50 mM ME (Fig. 4-6A). This stomatal closure was triggered by $HgCl_2$ effects on roots since we did not observe any effect of $HgCl_2$ in excised shoots (Fig. 4-6B). Low soil temperatures are known to inhibit root hydraulic conductivity and induce stomatal closure in aspen (Wan et al 1999). It is possible, that similarly to low root temperature (Chen et al 1983, Lee et al 1993) and drought (Zhang et al 1987, Lang et al 1994), $HgCl_2$ treatment triggered ABA synthesis which directly caused stomatal closure. This could explain the reason for a slow recovery of stomatal opening following the ME treatment. On the other hand, the lack of a significant effect of mercuric treatment on the stomatal conductance in excised shoots suggests that the significant route of water transport in the shoot tissues may be through the apoplast or that the channel-mediated water transport in the shoots may be absent.

The result of respiration experiment showed that 0.1 mM $HgCl_2$ did not significantly reduce root respiration during the initial hour at the time when the root water flow rate was significantly reduced (Figs. 4-1 and 4-2). This suggests that, in our experiment, the mercuric inhibition of root water flow was not due to metabolic inhibition. However, higher concentrations of $HgCl_2$ and those of longer duration, reduced root oxygen uptake (Fig. 4-7). The 0.1 mM $HgCl_2$ concentration used in our study caused a reduction of root respiration over time. However, the reduction in respiration rates was not paralleled by the reduction in the water flow rates. Additional evidence suggesting that the reduction of root water flow by mercury was not due to the inhibition of metabolism comes from the experiments designed to measure the activation energy for root water flow, in which the ascending plot for the 0.1 mM $HgCl_2$ treatment almost exactly overlapped with the descending one (Fig. 4-5). Also, the same concentration of mercury chloride had no effect

on the stomatal conductance in the excised shoots for at least 12 hours of the treatment (Fig. 4-6B). Our results suggest that the mercury-sensitive processes, likely those involving water channels, play an important role in regulating root hydraulic conductivity and, in effect, water relations in aspen. The observed low temperature sensitivity of the water transport in roots may be an important factor in the adaptation to winter conditions.

ACKNOWLEDGMENTS

We are grateful to Drs. VJ Lieffers, SM Landhäusser, and S Renault for their help with various aspects of this study and stimulating discussions. This study was funded by research grants from the Natural Sciences and Engineering Research Council of Canada and Sustainable Forest Management Network of Centres of Excellence.

Table 4-I. Effect of 0.1 mM $HgCl_2$ and temperature on the properties of xylem exudates

Sap		Reference	Descending	Ascending
Q_v at 25°C	Control	--	7.944 ± 0.801 a	3.665 ± 0.294 b
(m^3 s^{-1}) *10^{10}	$HgCl_2$	4.085 ± 0.902 a	2.205 ± 0.441 b	1.730 ± 0.385 b
Electrical	Control	--	670.83 ± 42.387 b	746.67 ± 65.167 a
conductivity (μS)	$HgCl_2$	660.33 ± 21.833 b	825.07 ± 38.224 a	800.33 ± 33.482 a
Osmotic	Control	--	-0.053 ± 0.0060 a	-0.059 ± 0.0129 a
potential (MPa)	$HgCl_2$	-0.058 ± 0.0014 a	-0.056 ± 0.0014 a	-0.057 ± 0.0023 a

The saps were collected only when the measurement temperature was at 25°C. Descending and ascending refer to decreasing and increasing temperatures, as explained in the text. The reference xylem sap was collected at 25°C before $HgCl_2$ was added.

Means ± SE (n=6) followed by different letters are significantly different at the 0.05 level.

Table 4-II. Properties of xylem saps collected from control roots and roots treated with $HgCl_2$ incubated in solutions containing Rhodamine B (RB)

Treatment	Q_v ($m^3\ s^{-1}$) $*10^{10}$	Electrical conductivity (μS)	Osmotic potential (MPa)	RB conc. ($\mu g\ ml^{-1}$) $*10^3$	C_e/C_b (%) $*10^2$
Control	6.12 ± 1.263 a	538.56 ± 22.076 b	-0.064 ± 0.0037 a	2.43 ± 0.205 b	1.22 ± 0.101 b
$HgCl_2$	2.87 ± 0.610 b	797.87 ± 54.658 a	-0.062 ± 0.0054 a	5.00 ± 1.106 a	2.50 ± 0.563 a

In the last column, C_e denotes RB concentration in the xylem sap, and C_b is RB concentration in the incubation solution.

Means ± SE (n=6) followed by different letters are significantly different at the 0.05 level.

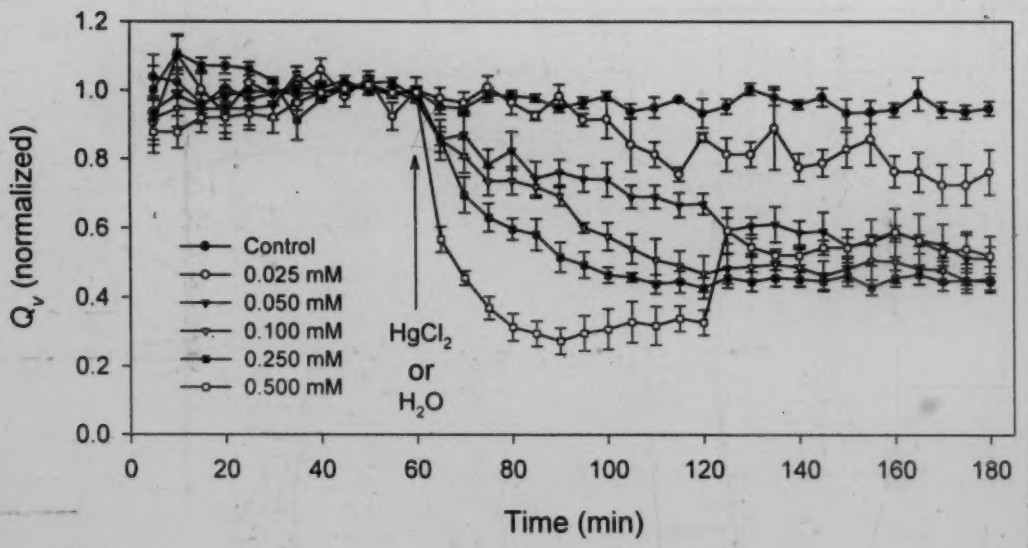

Figure 4-1. Dose response and time course of Q_V inhibition by mercury chloride. Q_V was normalized to the mean rate over the initial 30 min before $HgCl_2$ injection. The time of injection of $HgCl_2$ (H_2O for controls) is indicated by the arrows. Means ± SE are shown (n = 4).

Q_V was measured using the hydrostatic pressure method (Markhart et al 1979, Rüdinger et al 1994) with some modifications. A glass cylinder was inserted into a pressure chamber (PMS Instruments, Corvallis, OR, USA) and filled with half-strength Hoagland's solution, which was continuously stirred with a magnetic stirrer. A detopped root system was immediately sealed in the pressure chamber. The whole root system was immersed in the solution and surrounded with a copper coil, which was connected to a circulating cooler system (HAAKE F3, Berlin, Germany) to maintain the desired root temperature (± 0.1°C). A desired pressure was gradually applied with compressed air and maintained during the measurements. A graduated pipette was attached with a short piece of rubber tubing to the stem protruding through the stopper in a pressure chamber. Root flow rates of whole root systems were monitored for linearity for at least 30 min. and Q_V values are expressed in $m^3 s^{-1}$. J_V was calculated as a steady-state flow rate per unit root surface area and expressed as $m^3 m^{-2} s^{-1}$. Roots were assumed to be cylindrical and root surface area was calculated by multiplying the projected area, measured following computer scanning (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA), by π. In the previous experiment (Wan et al 1999), we found that Q_V in aspen was closely related to new root growth. Therefore, in the present study, J_V values are based on the new root surface area.

Dose response and time course of mercuric chloride treatment. Root systems were gradually pressurized to a constant pressure of 0.3 MPa. A stable Q_V was maintained for at least 30 min followed by injection of $HgCl_2$ with a syringe into the chamber to reach a required concentration. The Q_V was monitored during the following two hours. Distilled water was injected in place of $HgCl_2$ as a control. The stable mean Q_V values measured over the 30-min period before the $HgCl_2$ injection were used to normalize the treatment values.

Root respiration. Respiration was measured as oxygen uptake using a Clark-type electrode (Yellow Springs Instrument Co., Yellow Springs, OH). Intact roots were transferred to an airtight cuvette containing aerated half strength Hoagland's solution

which was continuously stirred with a magnetic stirrer and aerated every 30 min. The mean values from the first 30 min before injecting $HgCl_2$ were used to normalize the respiration rates following the treatments. Distilled water or different concentrations of $HgCl_2$ were added and oxygen uptake measured every 2 min.

The kinetics of water flow inhibition by mercuric chloride and its reversibility by ME. Root systems were gradually pressurized to a constant pressure of 0.3 MPa. When a stable Q_v was reached, $HgCl_2$ was injected with a syringe into the chamber to reach a final concentration of 0.1 mM. Q_v was monitored until a new stable flow rate was attained and then ME was injected into the chamber to provide a final concentration of 50 mM. The measurements of Q_v continued until another stable Q_v was reached. A control experiment was run in a similar manner, except that distilled water was injected in place of $HgCl_2$ solution.

Measurements of E_a. Root systems were immersed in half-strength Hoagland's solution and held in a constant pressure of 0.3 MPa with the temperature changing from 25°C to 4°C (descending) and back to 25°C (ascending) in 3°C steps for Arrhenius plot determinations. The temperature was monitored using a microprocessor thermometer with a fine wire type J-K-T thermocouple sealed into the pressure chamber through the rubber stopper. The compressed air was used for applying pressure in the chamber and the solution was continuously stirred with a magnetic stirrer. After the descending temperature series, the pressure chamber was opened and the solution aerated before continuing with the ascending temperatures.

The Arrhenius plots were obtained by plotting the logarithm of Q_v against the reciprocal of the absolute temperature and E_a was calculated from the slope of the whole curve of the descending plot. Before $HgCl_2$ addition, a Q_v value was measured at 25°C and used as a blank. Then, $HgCl_2$ was added into the solution to a final concentration of 0.1 mM based on the dose response data, and the temperature was lowered to 4°C and returned to 25°C in 3°C steps.

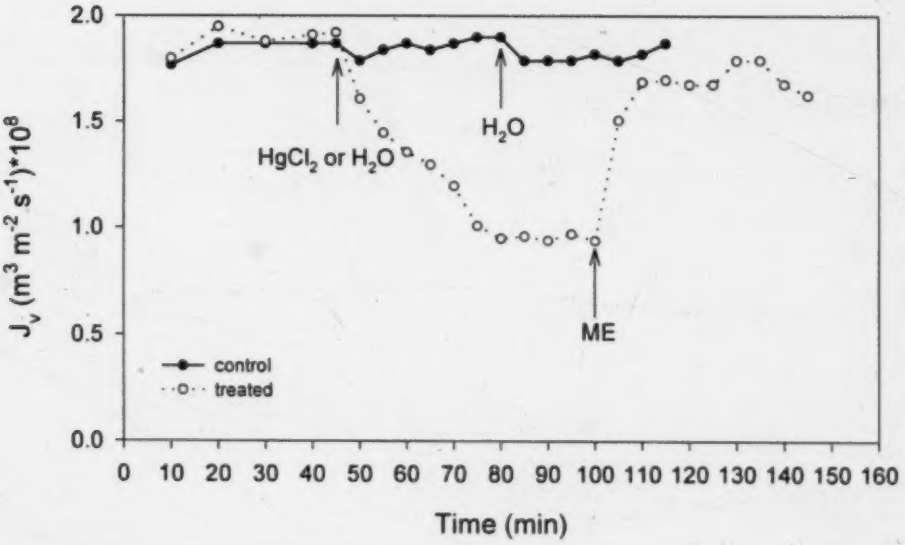

Figure 4-2. Volume flow density (J_V) in aspen roots treated with 0.1 mM $HgCl_2$ and 50 mM 2-mercaptoethanol (ME). Treatment (dashed line) is the mean of three seedlings; control (solid line) is the mean of two seedlings. Times of injections of $HgCl_2$ (H_2O for controls) and ME (H_2O for controls) are indicated by arrows.

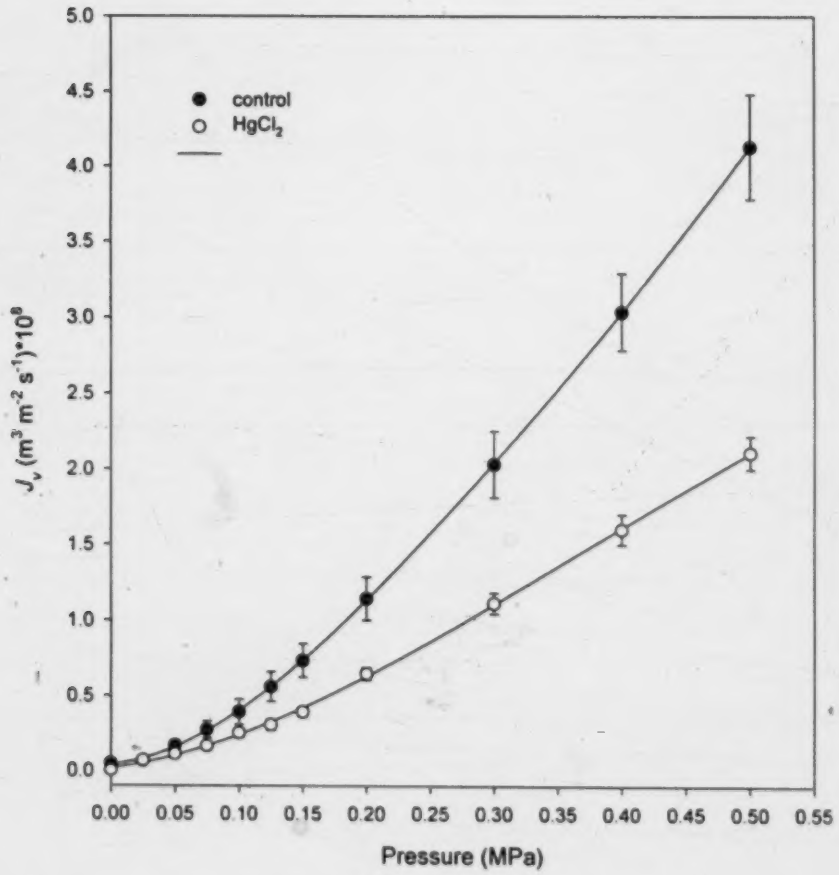

Figure 4-3. Pressure-flow relationship in control roots and roots treated with 0.1 mM $HgCl_2$. Means ± SE are shown (n = 6).

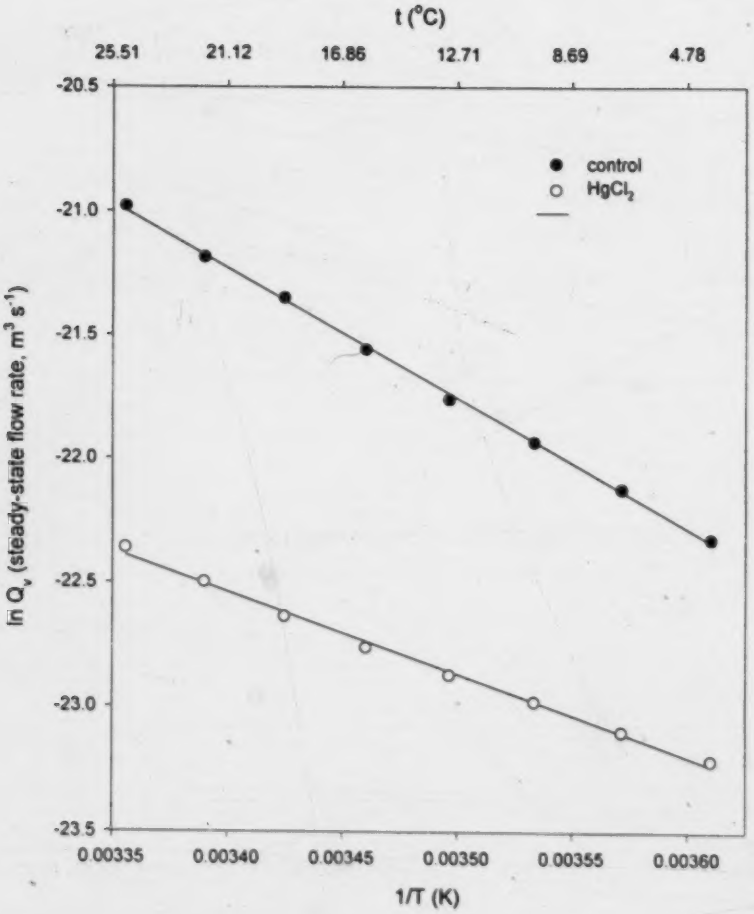

Figure 4-4. Temperature effects on water flow through aspen roots at constant hydrostatic pressure of 0.3 MPa and decreasing temperatures for control and $HgCl_2$-treated seedlings. Each curve is the mean of 6 seedlings from 6 repeated experiments.

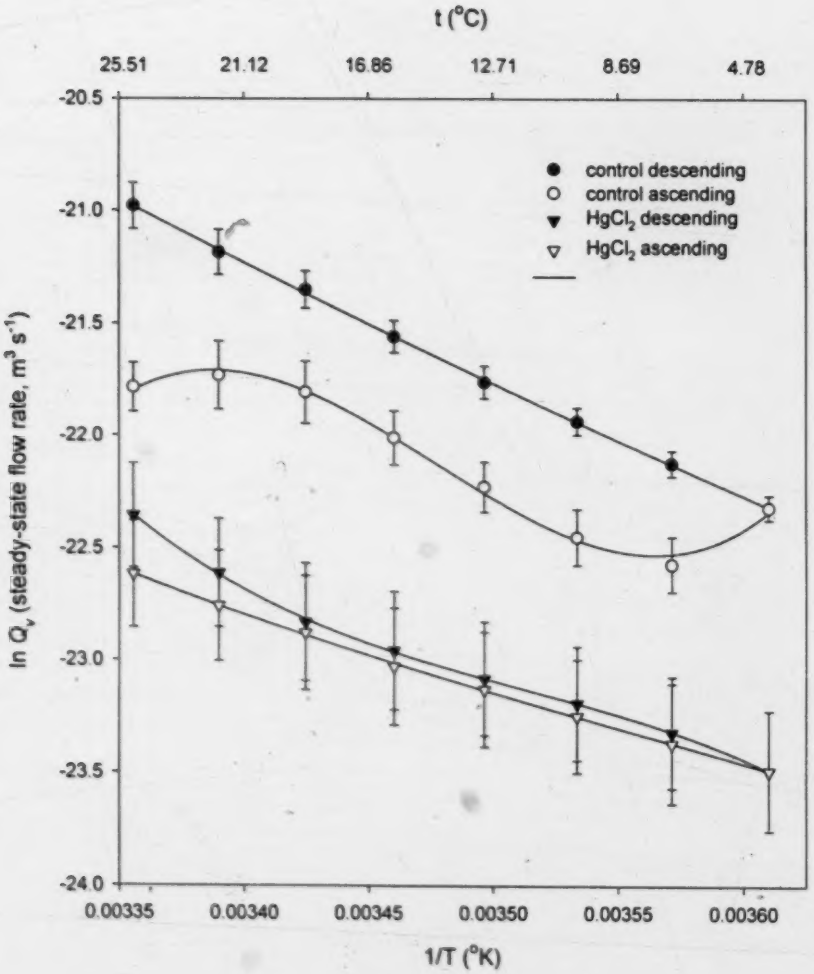

Figure 4-5. Temperature and $HgCl_2$ (0.1 mM) effects on water flow through aspen roots. Steady state water flow was continually measured in temperatures descending to 4°C followed by ascending temperatures to 25°C. Each curve is the mean of 6 seedlings from 6 repeated experiments.

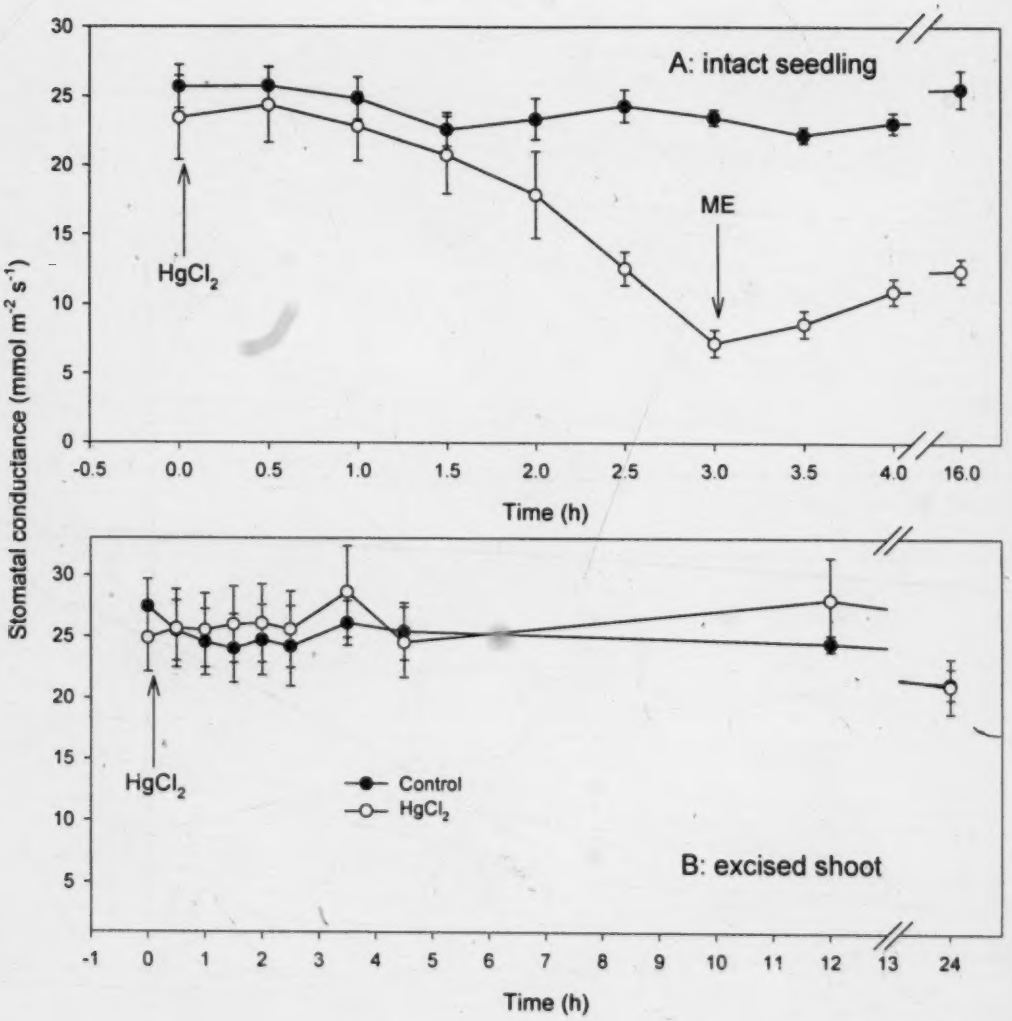

Figure 4-6. Effects of 0.1 mM $HgCl_2$ and 50 mM mercaptoethanol (ME) on leaf stomatal conductance (g_S) in (A) intact seedlings and (B) excised shoots. Arrows indicate the times when $HgCl_2$ and ME were added. Means ± SE (n = 6) are shown.

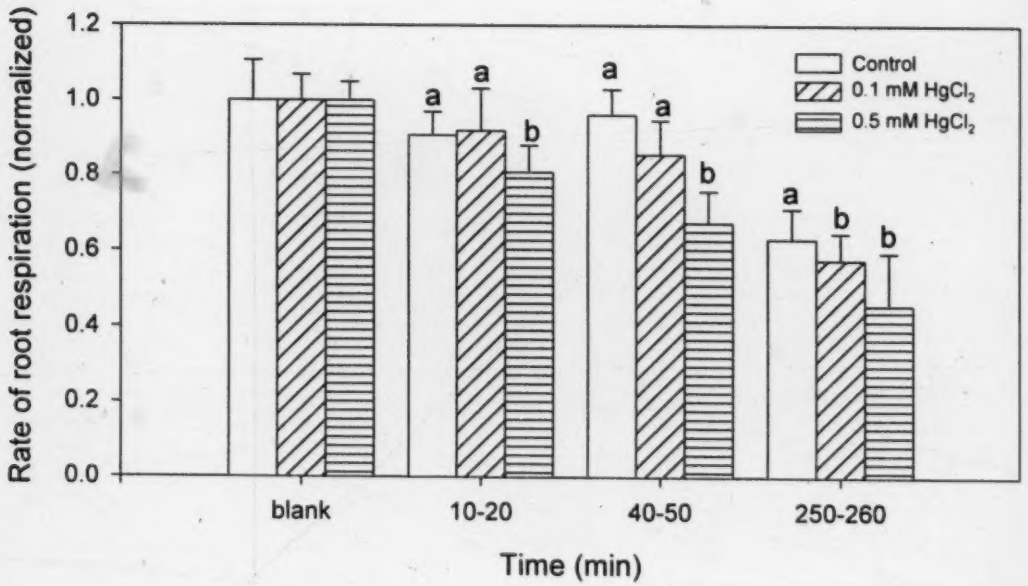

Figure 4-7. Effect of mercury chloride on root respiration. The mean values from the first 30 min before the $HgCl_2$ injection were used to normalize the respiration rates following the treatments. Bars with the different letters in the same group are significantly different at the 0.05 level. Means ± SE are shown (n = 4).

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CHAPTER FIVE[1]

Root water flow and leaf stomatal conductance in aspen (*Populus tremuloides*) seedlings treated with ABA

ABSTRACT

Exogenous ABA applied to the roots and excised shoots of aspen (*Populus tremuloides* Michx.) inhibited stomatal conductance proportionately to the applied concentration. Compared with intact seedlings, stomatal conductance in excised shoots was more sensitive to ABA. More than 10% of the ABA concentration applied to the roots was found in the xylem exudates of root systems exposed to 0.3 MPa hydrostatic pressure. A similar concentration of ABA applied to the excised shoots produced a faster and deeper reduction of stomatal conductance. ABA applied to the roots had no effect on root steady-state flow rate over the five-hour experimental period. Moreover, pre-incubating root systems of intact seedlings for twelve hours with ABA did not significantly reduce volume flow density. Similarly, ABA had no effect on root hydraulic conductivity and the activation energy of root water flow rates.

Key words: *Populus tremuloides* - Abscisic acid - Stomatal conductance - Root hydraulic conductivity- Water transport

INTRODUCTION

Water balance is largely determined by the rates of root water uptake, its transport through the plant and transpirational water loss. Under the conditions where water is readily available to the roots, root hydraulic conductivity and transpiration are the two

[1] A version of this chapter has been submitted for publication. Wan X, Zwiazek JJ 2000. Planta.

key elements controlling water balance. Abscisic acid (ABA) has been proposed to act as an essential mediator in triggering plant response to stress (Leung and Giraudat 1998). Numerous reports have demonstrated a powerful effect of ABA on the stomatal conductance of plants (Kriedemann et al 1972, Raschke and Hedrich 1985, Mansfield et al 1990, Trejo et al 1993). Abscisic acid can also increase (Glinka 1977, 1980, Freundl et al 1998 Quintero et al 1999) or decrease (Markhart et al 1979, Fiscus 1981, Davies et al 1982) root water flow rates. This variable effect is thought to depend on the rate of root water flow with an increasing effect at the low flow rates and a decreasing effect when the rates are high (Fiscus 1982, Davies et al 1982).

A comparative study using intact seedlings, detopped roots and excised shoots could help explain whether ABA regulates stomatal opening in plants through its effects on root hydraulic conductivity. Blackman and Davies (1985) and Zhang et al (1987) found in the split-roots experiments that the roots could sense soil water status and produce a signal, likely ABA, to trigger the corresponding shoot response. This work has triggered a heated discussion and studies on the relative importance of roots and shoots as the sensors of water stress (Kramer 1988, Passioura 1988, Boyer 1989). Subsequently, a number of reports showed a close relationship between the concentration of ABA in the xylem sap and the stomatal conductance when the roots were subjected to drying (Zhang and Davies 1990, Wartinger et al 1990, Tardieu et al 1992, Khalil and Grace 1993, Tardieu et al 1996). However, in addition to ABA, roots also produce other physiologically active substances, including cytokinins, which may affect stomatal opening (Bradford and Hsiao 1982, Davies et al 1986, Meinzer et al 1991). The role of these substances may be easily overlooked in the endogenous ABA studies.

In the present study, we applied ABA to the roots and measured its concentration in the xylem sap to determine how ABA affects stomatal conductance in the presence and absence of the root system. We studied the hypothesis that the effect of ABA on stomatal conductance in plants is exerted directly through its effect on stomatal opening rather than indirectly by reducing the supply of water to the shoots and that this effect is

modulated in the presence of roots. Therefore, we compared the effects of ABA applied to the roots and excised stems and we examined the effects of ABA on root water flow properties in aspen (*Populus tremuloides* Michx.) seedlings.

MATERIALS AND METHODS

Plant material. Aspen (*Populus tremuloides* Michx.) seedlings were grown in a growth chamber (Controlled Environments Inc. Winnipeg, MB, Canada) from seed collected near Whitecourt, AB, Canada. Seeds were germinated in Petri dishes and one day after germination the seedlings were transferred to styrofoam containers with sand and grown in the greenhouse for two months. After two months, the roots of seedlings were gently washed free of sand with tap water and transferred to solution culture containing half-strength modified Hoagland's solution (Epstein 1972). The seedlings were grown in solution culture for one month in the growth chamber under the following conditions: 16-h photoperiod with 260 μmol m^{-2} s^{-1} photosynthetically active radiation at the seedling level, 20/16°C (day/night) temperature and a constant RH of approximately 65%. The Hoagland's solution was continuously aerated and replaced every two weeks.

Root water flow. Steady-state flow rates of the whole root systems (Q_v) were measured using the hydrostatic pressure method as previously described (Wan and Zwiazek 1999). A glass cylinder was inserted into a pressure chamber (PMS Instruments, Corvallis, OR, USA) and filled with half-strength Hoagland's solution, which was continuously stirred with a magnetic stirrer. A detopped root system was immediately sealed in the pressure chamber. The whole root system was immersed in the solution and surrounded with a copper coil, which was connected to a circulating cooler system (HAAKE F3, Berlin, Germany) to maintain the desired root temperature (± 0.1°C). A desired pressure was gradually applied with compressed air and maintained during the measurements. A graduated pipette was attached with a short piece of rubber tubing to the stem protruding through the stopper in a pressure chamber. Root flow rates of whole root systems (Q_v) were monitored for linearity for at least 30 min. and Q_v values are expressed in m^3 s^{-1}.

To determine the effect of ABA on Q_v, detopped root systems were sealed in the pressure chamber and gradually pressurized to a constant pressure of 0.3 MPa. When a stable Q_v was reached, ABA solution of *cis-trans* isomers from Sigma (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) in 0.1% ethanol was injected with a syringe into the chamber to reach a final concentration of 5×10^{-5} M. Q_v was monitored for the next five hours. A control experiment was run in a similar manner, except that 0.1% ethanol solution was injected in place of ABA solution. Another group of intact seedlings was pre-incubated for twelve hours with 5×10^{-5} M ABA or 0.1% ethanol (control). These seedlings were excised and the root systems were used for Q_v measurements. Roots were assumed to be cylindrical and root surface area was calculated by multiplying the projected area, measured following computer scanning (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA), by π. J_v was calculated as a steady-state flow rate per unit root surface area and expressed as $m^3\ m^{-2}\ s^{-1}$.

Hydraulic conductivity (L_p). Roots were immersed in half-strength Hoagland's solution (with 0.1% ethanol for control, with 0.1% ethanol and ABA for treatment) in a pressure chamber. The pressure increased every 40 min from 0.1, 0.2, 0.3, 0.4 and 0.5 MPa. The volume flow density (J_v) was calculated as Q_v per whole root and plotted against hydrostatic pressures. To minimize variability, the Q_v at 0.3 MPa in half-strength Hoagland solution was measured for a half hour and then the similar Q_v values were paired, one used for control and another for treatment. Therefore the data were analyzed with paired *t*- test. L_p (root hydraulic conductivity) was calculated from the slope of the curve between 0.1 and 0.5 MPa where the relationship between pressure and J_v ($mm^3\ sec^{-1}\ root^{-1}$) was linear, and it is expressed in $mm^3\ sec^{-1}\ MPa^{-1}\ root^{-1}$.

Arrhenius plots. Root systems were immersed in half-strength Hoagland's solution (with 0.1% ethanol for control, with 0.1% ethanol and ABA for treatment) and held in a constant pressure of 0.3 MPa with the temperature changing from 25°C to 4°C in 3°C steps for Arrhenius plot determinations. The temperature was monitored using a

microprocessor thermometer with a fine wire type J-K-T thermocouple sealed into the pressure chamber through the rubber stopper. Compressed air was used for applying pressure in the chamber and the solution was continuously stirred with a magnetic stirrer. The Arrhenius plots were obtained by plotting the logarithm of Q_v against the reciprocal of the absolute temperature and the activation energy (E_a) was calculated from the slope of the whole curve of the plot.

Root anatomy. Freehand cross-sections of primary roots of hydroponically grown aspen were made at distances from the root tip of 20, 40, 60, 80 and 100 mm. Sections were stained for 1 h with 0.1% berberine hemisulfate and subsequently for 45 min with 0.5% toluidine blue O (w/v) as described by Freundle et al (2000). After being rinsed with distilled water to be freed of background blue color, the sections were observed under an epifluorescence microscope (Nikon, Japan; excitation/emission, 365/395 nm).

Stomatal conductance. Intact seedlings were transferred to 600-ml tall beakers containing aerated half-strength Hoagland's solution in a growth chamber under environmental conditions described for growth. In another experiment, excised shoots were used instead of intact seedlings. The leaves were removed from the lower part of stem and two days later the shoots were immersed in half-strength Hoagland's solution and excised at the root collar. The cut end of the shoot was immersed in a vessel containing the nutrient solution and placed in the growth chamber. A blank g_S measurement was conducted before adding ABA. ABA was dissolved in 95% ethanol and diluted with distilled water to a required final concentration. For controls, 0.1 % ethanol was used in place of the ABA solution. Measurements of g_S were conducted on the uppermost fully expanded leaves with a steady-state porometer (LI-1600, Li-Cor Ltd., Lincoln, NE, USA). All measurements were conducted during the light period.

ABA analysis. Roots were immersed in half-strength Hoagland's solution (with 5×10^{-5} M ABA in 0.1% ethanol for treated and 0.1% ethanol for control plants) in a pressure chamber. The pressure increased gradually to 0.3 MPa and was held for three hours.

Then, samples of xylem exudates were collected to measure ABA concentration. ABA analysis followed the methodology used by Markhart et al (1982). The xylem exudates were filtered through a 0.45 μm Millipore filter before injecting into the high-pressure liquid chromatograph (HPLC) equipped with the 254 nm UV detector. ABA was separated by an LC-18 column (Supelcosil™) using methanol:H_2O:acetic acid (50:50:1, by volume) solvent system and a flow rate of 0.8 ml min^{-1}. Standard curves, with the treating ABA solution, of peak area versus concentrations were linear between 5×10^{-5} M to 5×10^{-7} M and were used to determine ABA concentration in xylem exudates. The peak areas were calculated with a Hewlett-Packard HP 3396A integrator.

Water potential measurements. Shoot water potentials were measured in intact seedlings and excised shoots using a Scholander pressure chamber as described previously (Wan et al 1999).

Reagents. All reagents were of the highest available grade and were purchased from Sigma.

Statistical analysis. The data are presented as the means of at least six replicates (seedlings). The results were analyzed by ANOVA and with Duncan multiple comparison for the effects of ABA on stomatal conductance and root water flow over time using SAS 6.12 software package (SAS Institute Inc., Cary, NC, USA). A two-tailed *t*-test was performed for analyses of ABA concentration in the exudates (Fig. 5-2) and the activation energy (Fig. 5-6), a paired *t*-test for comparison of root hydraulic conductance (Fig. 5-5). All statistically significant differences were tested at the 0.05 level.

RESULTS

Anatomy. Aspen roots possessed tri-arch xylem in the primary roots. In most roots, lateral branching took place at a distance greater than 100 mm from the root tip and originated in the pericycle opposite xylem ridges. There was no exodermis structure present in the

roots. The roots developed a primary endodermis with the Casparian bands in the radial cell walls at distance greater than 20 mm from the root tip (Fig. 5-1A, B). At the distance greater than approximately 60 mm, fluorescent suberin deposits covered whole outer surfaces of the cell walls in the endodermis. The Casparian bands in the endodermis were not continuous. The passage cells present in the endodermis opposite xylem ridges lacked suberin deposits (Fig. 5-1C).

HPLC analysis of ABA. Fig. 5-2 shows an HPLC separation of 5×10^{-5} M *cis-trans* ABA standard solution (A), and root exudates with (B) and without (C) ABA added to the roots. ABA concentrations in exudates of roots treated with ABA averaged $5.16\times10^{-6} \pm 0.658\times10^{-6}$ M (n = 4), that equals 10.3% of the *cis-trans* ABA concentration present in the root solution. An ABA peak was absent from the control root exudates (Fig. 5-2C) analyzed by HPLC.

Effects of ABA on g_S. ABA added to the root medium reduced g_S in intact aspen seedlings and excised shoots (Fig. 5-3). In intact seedlings treated with 5×10^{-5} M and 10^{-4} M ABA, g_S declined approximately 15% after 4 h (Fig. 5-3A). After 24 h, g_S decreased by 29% and 36% in 10^{-4} M and 5×10^{-5} M ABA treatments, respectively. The lower, 10^{-5} M ABA was effective in g_S reduction only after 24 h of treatment (Fig. 5-3A). Over the experimental period, no significant changes in g_S were detected in control seedlings ($p = 0.726$).

The concentrations of exogenous ABA needed to reduce g_S were considerably lower in the excised shoots compared with the intact seedlings (Fig. 5-3). In plants treated with 5×10^{-6} M and 10^{-5} M ABA, g_S declined in 90 min. by about 20% and 34%, respectively (Fig. 5-3B). After 24 h, g_S decreased by 41% in 5×10^{-6} M and 54% in 10^{-5} M ABA compared with untreated control. The lower, 10^{-6} M concentration did not significantly reduce g_S (Fig. 5-3B).

Effects of ABA on root water flow. ABA treatment did not have an effect on Q_v during the 5-h experimental period (Fig. 5-4). Moreover, pre-incubating root systems of intact seedlings for twelve hours with 5×10^{-5} M ABA did not significantly affect J_v. The control and ABA-treated roots had J_v values of $5.87 \pm 0.401 \times 10^{-8}$ m^3 m^{-2} s^{-1} and $5.02 \pm 0.303 \times 10^{-8}$ m^3 m^{-2} s^{-1} (n=6 ± SE), respectively.

Pressure-flux curves showed a linear relationship between 0.1 and 0.5 MPa in both control and ABA-treated roots (Fig. 5-5). There was also no difference in L_p values which measured 1.88 ± 0.569 and 1.89 ± 0.572 mm^3 sec^{-1} MPa^{-1} $root^{-1}$ (n=6 ± SE) in ABA-treated and control roots, respectively.

Both control and treated roots had linear Arrhenius plots for Q_v (Fig. 5-6). There existed variation in Q_v between control and treatment, however their activation energies (E_a) were similar and measured 8.73 ± 0.430 and 8.31 ± 0.249 kcal mol^{-1} (n=6 ± SE) in ABA-treated and control roots, respectively.

Shoot water potentials. The shoot water potentials measured -0.63 ± 0.036 MPa (n =6 ± SE) in intact plants and -0.41 ± 0.015 MPa (n=6 ± SE) in excised shoots.

DISCUSSION

The presence of ABA in the root xylem exudates demonstrates that, in our study, substantial quantities of ABA added to the root medium were carried with the transpiration stream to the shoot. Similar results have been reported previously (Markhart 1982, Freundl et al. 1998). The concentration of ABA in the xylem sap is believed to be modulating stomatal conductance (Tardieu and Davies 1992, Liang et al. 1996) and transpiration rate (Jarvis and Davies 1997). In the 5×10^{-5} M ABA treatment, the concentration of ABA in the xylem sap was $5.16 \pm 0.658\times10^{-6}$ M, similar to the concentration used for the excised shoots effective in reducing stomatal conductance (Fig. 5-3). ABA transport out of the root systems is a function of volume flux (Fiscus et al.

1982). The uptake of ABA from the medium by solvent drag may increase the concentration of ABA in the xylem by water uptake (Freundl et al. 1998). In our study, the water potential difference between the shoots and root medium was about -0.6 MPa. Therefore, it is possible that the concentration of ABA in the xylem sap of intact seedlings treated with ABA was even higher than that measured in the root exudates expressed at 0.3 MPa pressure.

The composite transport model, proposed by Steudle (1994), provides a good description about water and solutes radial movement through roots. In our study, water and solutes could freely enter roots by the apoplastic pathway before reaching the endodermis that is believed to act as a barrier to water and solutes. However the endodermis may not completely block water and solute transport for the following reasons. (1) The formation of Casparian bands is a dynamic process, some parts may be more permeable to water and solutes than other parts due to their maturity. In aspen roots some endodermal cells were not suberized (Fig. 5-1C). (2) The origin of lateral roots from the pericycle creates gaps that provide a bypass route for water and solutes. (3) The Casparian strips are a perfect barrier to large molecules, such as PTS or Rhodamine B (Skinner and Radin 1994, Zimmermann and Steudle 1998, Wan and Zwiazek 1999), whereas smaller molecules, like ABA, may sneak through the bands (Freundl et al 1998, 2000, Schreiber et al 1999). (4) ABA is an endogenous substance in plants, and it has been regarded as a messenger from roots to shoots (Davies et al 1994). In the rhizosphere, there is an amount of ABA that may be taken up by root systems (Hartung et al 1996). Therefore root systems may have evolved the capacity to take up ABA from root media and transport it through roots via both apoplastic and symplastic routes.

Water flow in aspen roots was more dependent on temperature than a viscous flow of water across a porous system (Fig. 5-6). This suggests that the symplastic (cell-to-cell) water flow in aspen roots is dominant. On the other hand, 10% of root medium ABA concentration was detected in the xylem sap suggesting apoplastic flow. The reflection coefficient of aspen root for ABA flow calculated from our data based on the equation

introduced by Freundle et al (1998) is above 0.8. This value is higher than that in maize (Freundle et al 1998). A large amount of ABA can cross the endodermis, however, the apoplastic pathway may not be dominant since the reflection coefficient is still high. In addition, the symplastic transport of ABA through roots may not be completely excluded.

The extrapolation of the pressure/flow curve shown in Figure 5-5 has an intercept with the pressure axis at a positive value of J_v. This is different from the type of curves usually obtained (Fiscus 1981, Fiscus et al 1982). The incidence may be due to ethanol treatment. Ethanol reduced the osmotic concentration of root xylem exudates (Markhart et al 1979). This change could result in a sharp response in water flow at low pressures according to Fiscus's theory (Fiscus 1975).

In the present experiment, ABA application to aspen roots did not alter the rate of root water flow as reported for sunflower (Glinka 1977) and soybean (Markhart et al. 1979). ABA can have different effects on root water flow depending on hydrostatic pressure gradients (Markhart et al. 1979). ABA increased root water flow rate when the rate was initially low (Davies et al. 1982, Markhart 1984) due to its stimulating effect on ion accumulation (Fiscus 1981). In our study, root water flow rate of ABA-treated plants did not change at low or at high flow rates as evidenced by the lack of effect on L_p and on the Arrhenius plot (Figs. 5-5, 5-6). Additionally, ABA did not significantly affect Q_v, J_v, L_p and E_a of aspen roots. The results suggest that ABA applied to the roots affects stomatal conductance directly by triggering stomatal closure rather than indirectly through changes in plant water balance due to the inhibition of root water flow like that reported for cold roots (Wan et al. 1999).

It appears that the differences in stomatal response of excised shoots and intact seedlings may not be due to ABA concentration differences. Higher xylem concentrations of ABA applied to the rooted seedlings exerted less effect on the stomatal conductance than in the excised shoots. The sensitivity of stomata to ABA could also be due to the differences in plant water status (Tardieu and Davies 1992). We cannot completely exclude that stem

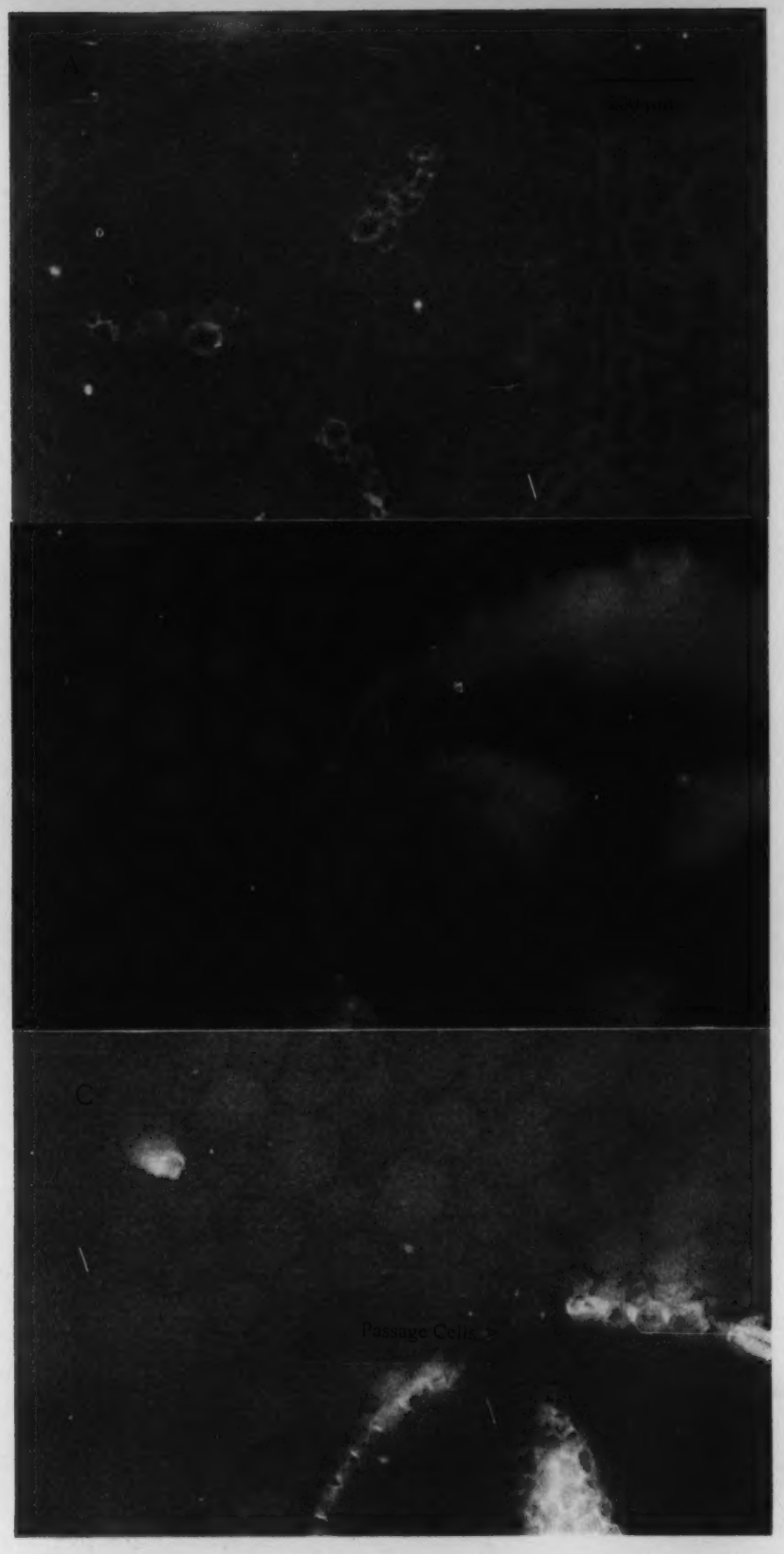
cutting made the stomata more responsive to ABA treatments. However, in the intact aspen seedlings, shoot water potential was significantly lower than that in the excised shoots. Therefore, we expected the stomata in the intact plants to be more sensitive to ABA than those in the excised shoots (Tardieu and Davies 1992). Our results suggest that the presence of roots modulates stomatal response. It is evident from this and other studies (Zhang et al. 1987, Davies and Zhang 1991, Davies et al. 1994) that ABA can act as a signal substance to regulate stomatal aperture. However, the differential effects of ABA on stomatal conductance between the intact seedlings and excised shoots suggest the possibility that other signals which originate in the roots may modify stomatal response to ABA. It has been proposed that the interactions between ABA and cytokinins control stomatal movement in plants (Bradford and Hsiao 1982). It is possible that the higher sensitivity of stomata to ABA in excised aspen shoots compared with that in rooted plants could be explained by the absence of the source of cytokinins.

In summary, our results showed that exogenous ABA applied to the roots inhibited stomatal conductance in aspen seedlings but had no effect on the root water flow. The concentration of ABA needed to reduce stomatal conductance in rooted plants was significantly higher compared with excised shoots. These results suggest that the roots modulate stomatal response to ABA.

ACKNOWLEDGEMENTS

We gratefully acknowledge research funding from the Natural Sciences and Engineering Research Council in the form of a research grant to JJZ.

Figure 5-1. Freehand cross-sections of aspen primary roots at various stages of development. (A) 20 mm from root tip, there is no Casparian bands. (B) 40 mm from root tip, Casparian bands are seen as light strips in the radial walls. (C) 80 mm from root tip, Suberin deposits are around the cells in endodermis, except the passage cells.


A
C
Passage Cells

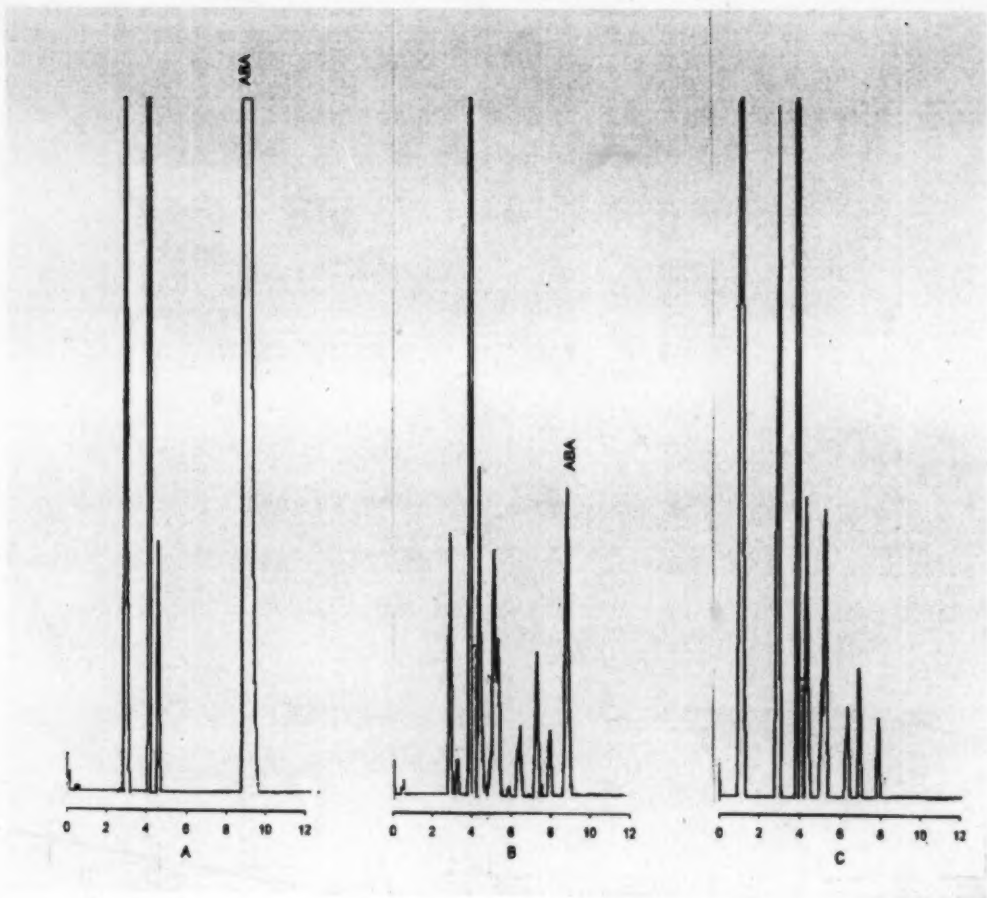


Figure 5-2. HPLC separation of 5×10^{-5} M *cis-trans* ABA treating solution (A) and xylem exudates of roots treated with ABA (B) and control (C).

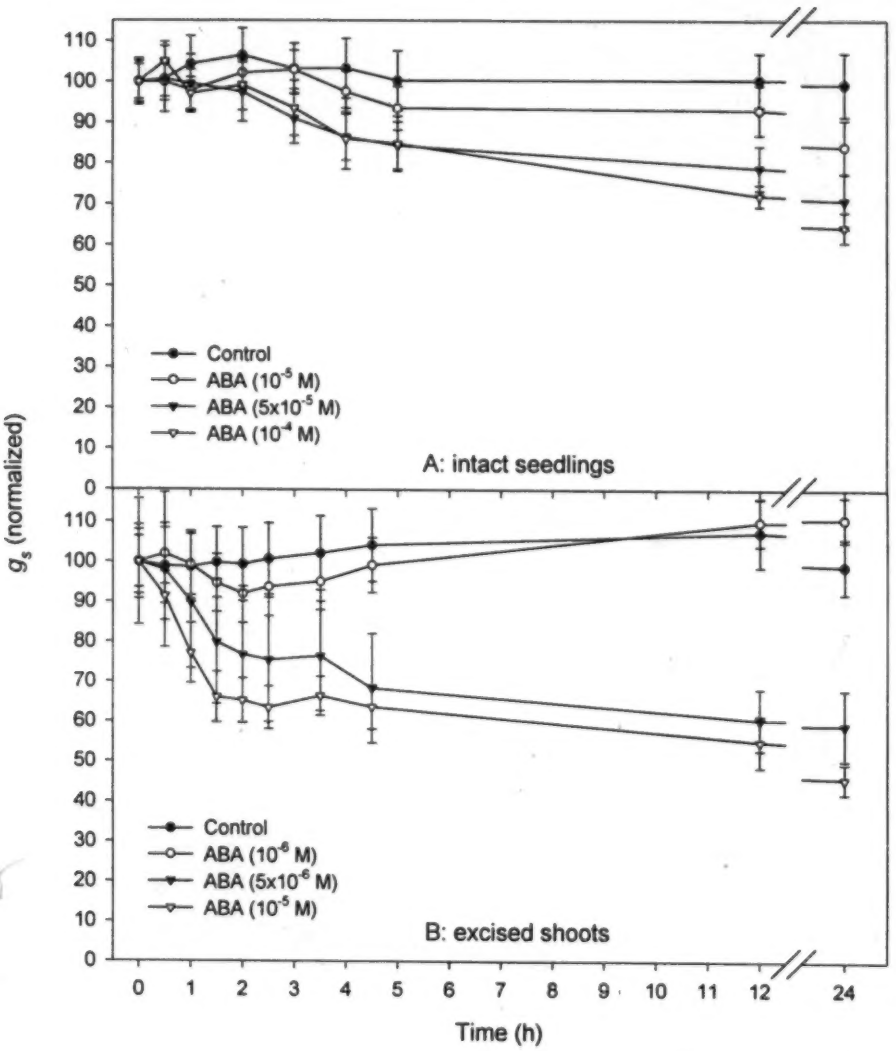


Figure 5-3. Effects of ABA on leaf stomatal conductance (g_S) in (A) intact seedlings and (B) excised shoots. The values for g_S were normalized to those before ABA and ethanol were added (time zero). Means ± SE (n = 6) are shown.

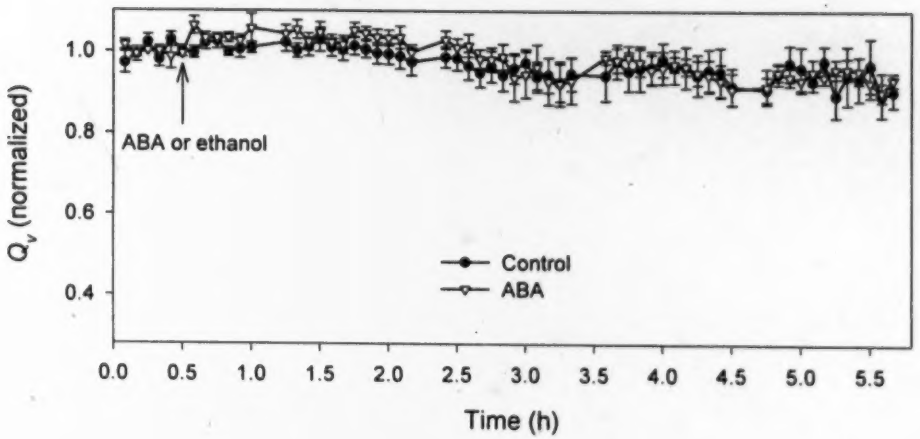


Figure 5-4. Water flow rate (Q_V) in aspen roots treated with 5×10^{-5} M ABA. Q_V was normalized to the mean rate over the initial 30 min before ABA or ethanol were added to treated and control roots, respectively. Arrows indicate times of addition of ABA (and ethanol for controls). Means ± SE are shown (n = 6).

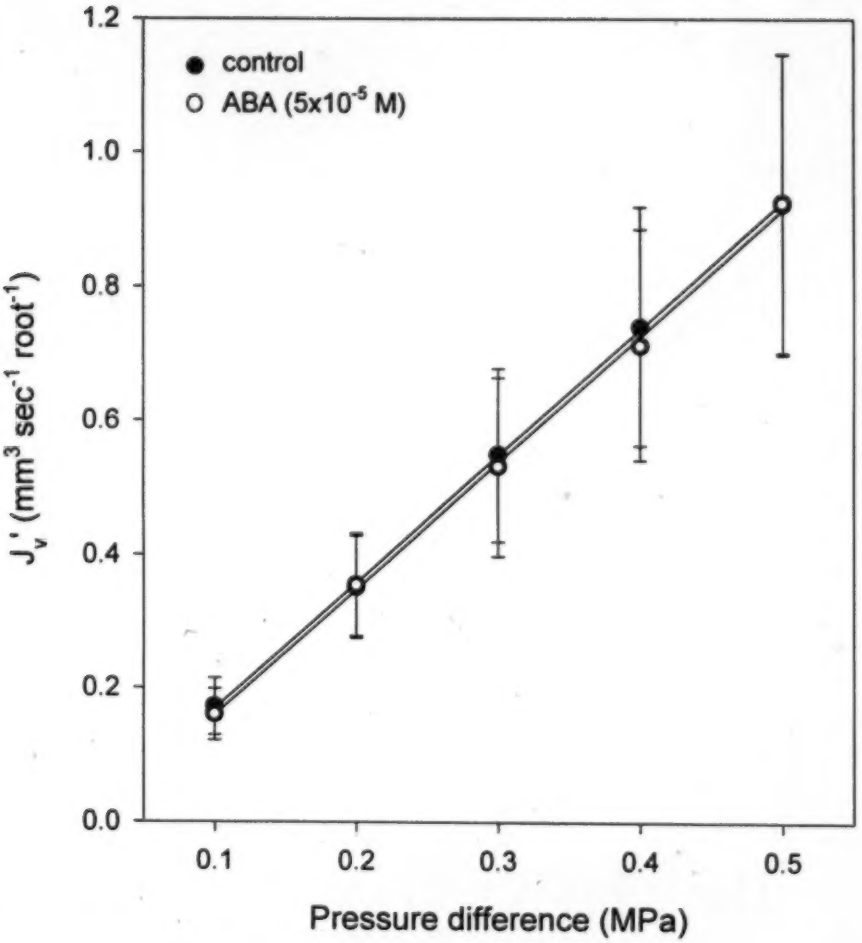


Figure 5-5. Pressure-flow relationship in control roots and roots treated with 5×10^{-5} M ABA. The ABA treated and control root samples of similar Q_v values at 0.3 MPa hydrostatic pressure were paired and analyzed by the paired t-test. Means ± SE are shown (n = 6).

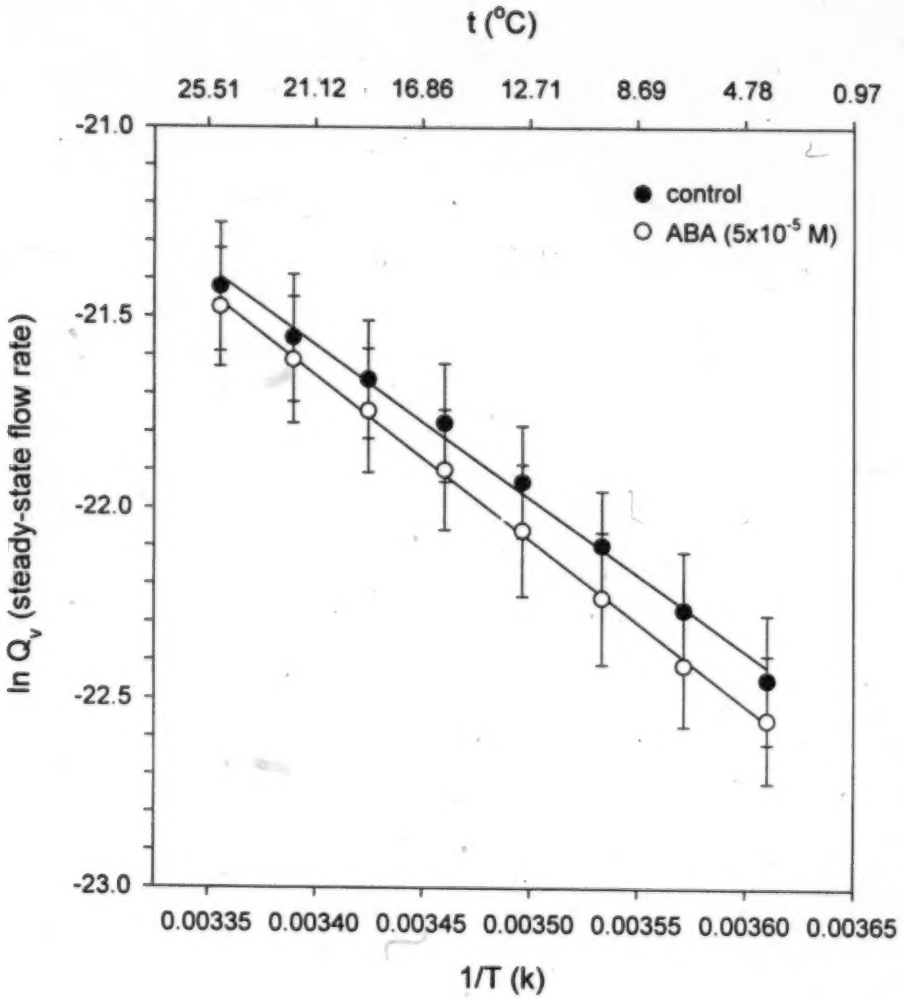


Figure 5-6. Temperature effects on water flow through aspen roots at the constant hydrostatic pressure of 0.3 MPa and decreasing temperatures. Means ± SE are shown (n = 6).

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CHAPTER SIX

General Discussion and Conclusions

Low soil temperature is an important ecological factor in the boreal forest. Cold soils are found in many places in the boreal forest even during warm summer months (Tryon and Chapin 1983, Hogg and Lieffers 1991). Low soil temperatures reduce plant growth. The reduced growth could be a consequence of decreased uptake of water and nutrients or (and) altered biochemical processes within the plant. In the present study, I investigated the effect of low root temperature on water flow in aspen seedlings.

The results of my study showed that low root temperature reduced growth (root, shoot and leaf), lowered shoot water potential, stomatal conductance, and photosynthesis in aspen. Reduced root growth and root hydraulic conductivity caused a decrease in root water flow rate. The reduced water flow was closely correlated with reduced shoot growth and leaf expansion (Chapter 2). Interestingly, hydraulic conductance in Engelmann spruce (*Picea engelmannii*) and white spruce (*Picea glauca*) were relatively unaffected by soil temperatures as low as 8°C (Kaufmann 1975, Delucia 1986) or 5°C (our unpublished data). Although aspen grows in similar sites in the boreal forest as white spruce, its root hydraulic conductivity was significantly reduced when soil temperature declined below 15°C (Chapter 3). The values were $9.22 \pm 0.465 \times 10^{-8}$ m s^{-1} MPa^{-1} for 20°C, $6.78 \pm 0.547 \times 10^{-8}$ m s^{-1} MPa^{-1} for 15°C, and the difference was statistically significant ($P < 0.001$). Aspen transpiration demands in winter are very low, while the transpiration of evergreen conifers can be relatively high on mild and sunny winter days (Kramer 1983). To avoid winter drought stress, evergreen northern conifers should facilitate relatively high water uptake ability from cold soils, even through their suberized roots (Kramer 1983).

In the experiments of Chapter 3, chilling inhibition of root water flow was found not fully reversible by higher temperatures. However, the inhibition was not because of membrane damage. In the another experiment (Chapter 4), when mercuric chloride was added to the root medium, root water flow was partly inhibited by mercuric chloride, and root flow rates that were reduced by low temperatures could be fully reversed at higher temperatures. It is noteworthy that, contrary to previous reports (Chrispeels and Agre 1994, Macey 1984, Verkman et al 1996), the mercury sensitive root water transport processes were temperature-sensitive (Chapter 4). Perhaps, perennial woody plant species possess a different system from that of shorter-lived plants. It is possible that low temperature induced conformational changes of the water channel proteins affected their gating function as happened in ion transport through membranes (Hladky and Haydon 1972). However, none of the cloned plant or animal aquaporins has been reported to be gated by hydrostatic or osmotic pressure gradients (Maurel 1997). It should be noted the present study is the first comprehensive investigation of water channels in perennial woody plants. The limitation of water uptake, when temperatures decline at the end of the growth season, is among the traits used by plants to prepare for winter. Therefore, perennial plants may have different adaptive mechanisms to the seasonal change in temperature from homoiothermic (warm-blooded) animals or annual plants. Homoiothermic animals can maintain a constant inner temperature, while the annual plants may not encounter harsh winter temperatures.

Stomatal conductance responded to the reduced root water flow at low root temperatures, and the response was proportional to the extent of reduction (Chapter 3). There appears to be a threshold water level required to produce stomatal response. Above this water level, guard cells obtain water from the neighboring cells to maintain the stomatal opening, probably by osmotic regulation. This preserves gas exchange. If water levels decline below the threshold, the guard cells reduce their solute content and deflate, and the stomata close to prevent excessive water loss. In the former case, increasing the water supply, by rising root temperature, immediately swells the guard cells and widens stomatal aperture more than before stress. This likely takes place before osmotically-

active solutes are exported from the guard cells and their osmotic potentials increase resulting in a decline in turgor to the pre-stress level. Another interpretation includes the involvement of certain metabolic processes in initiating stomatal closure and preventing opening (Raschke 1987, Assmann and Shimazaki 1999). Therefore, re-supplying the leaves with water could not immediately restore stomatal opening to the original level. Although root water flow in aspen was chilling sensitive, the slow recovery of stomatal conductance at 25°C after the 4°C treatment (Chapter 3 Fig. 3-5) could be a result of the metabolic mechanism of stomatal movement. The water flow rate, after chilling treatment, was still fairly high at 25°C (Chapter 3 Fig. 3-3).

In drought stress, one of the functions of ABA is the induction of stomatal closure. Similarly to dehydration stress, the impacts of low soil temperature on plants include a reduction of water uptake. There is evidence that ABA is produced by roots in drying soil, moves in the transpiration stream and accumulates near the guard cells (Cornish and Zeevaart 1985, Zhang et al 1987). However no bulk increase in ABA was detected in aspen xylem sap in our experimental system when the roots were subjected to low soil temperatures (results not shown). The results cannot exclude that ABA plays a role in mediating stomatal aperture by redistributing among organs and cells (Raschke 1987, Hartung and Davies 1991). Exogenous ABA application experiments showed that ABA rapidly induced stomatal closure in aspen leaves. Stomata responded differentially to the root and shoot xylem ABA applications. The results suggested that, perhaps, another internal signal may be needed to interact with ABA to control stomatal aperture. It is noteworthy that, contrary to several other studies (Markhart et al. 1979; Davies et al 1982; Glinka 1977), ABA did not affect the properties of water transport through aspen roots.

CONCLUDING REMARKS

This research has shown that soil temperature is crucial for new root growth and root hydraulic conductivity in aspen. In spring, root pressure needs to build up to refill

embolisms produced by freeze-thaw cycles in a harsh winter (Tyree and Sperry 1989, Sperry 1993). The capacity to refill embolisms is correlated with dieback occurrence in some deciduous species (Sperry 1993). Roots are usually the place of the greatest hydraulic resistance in plants (Kramer 1983). However, the cavitation of xylem elements in shoots can result in even higher tissue resistance to water flow. Cavitation may be caused by drought and by freezing and thawing of xylem sap when it is under tension (Sperry 1995). If roots grow before the bud break and shoot expansion, root pressure in deciduous species may build up, and eliminate cavitation. If the cold soil limits new root growth, then the cavitation produced by freezing and thawing may be further exacerbated. The causes of high tree mortality in many boreal stands of aspen are largely unknown. Perennial plants of the boreal forest experience freezing which may result in embolisms of the water-conducting xylem elements. Embolisms in conduits increase resistance to water transport. Soil temperature rises in spring and promotes new root growth required to increase root pressure and fill the xylem elements with water. However, low soil temperature, which is commonly present in many boreal forest sites, can limit this recovery and aggravate the embolism problem in aspen trees at the time of bud burst and shoot expansion. Low soil temperature and relatively high air temperature can upset plant water balance and contribute to forest decline.

In the prevailing concept of water transport, water absorption is a passive process that does not directly require metabolic energy. Osmotically driven absorption is an indirect energy-demanding process. In this process, roots function as osmometers. Water channels are also often regarded as pores providing a passive pathway for water transport. The root systems merely provide an absorption surface. The same absorption capacity of dead roots as live ones is strong evidence to support the concept. In those experiments, roots were usually killed by treatments such as ethanol or hot water. However, treating the roots with agents such as ethanol, hot water, etc. results in their loss of control over water and ion absorption due to the loss of cell membrane selective permeability. Therefore, the results of such experiments should not be used to support the hypothesis that roots act merely as a passive absorbing surface. When the roots are killed by hot water or ethanol,

the water flow rates through the roots are often higher compared with those prior to the treatment. (Smit and Stachowiak 1988, Freundl et al 1998, Wan and Zwiazek unpublished data).

Another line of evidence for an involvement of metabolic energy in water transport is that when roots are treated with respiration inhibitors such as azide, cyanide, both osmotic and passive absorptions are drastically reduced (Zhang and Tyerman 1991, Kamaluddin et al 2000, Wan and Zwiazek unpublished data). This phenomenon was attributed to increased resistance of water transport, as well as reduced accumulation of ions in osmotic absorption. The unresolved question is how the metabolic inhibitors influence flow resistance if the absorption of water does not directly involve energy consumption as assumed (Weatherley 1982, Kramer 1983). The response of root water flow to respiration inhibitors is very rapid. Under transpiration or hydrostatic pressure, the osmotic gradient contributes an almost negligible part of the driving force for water transport through roots. Membrane synthesis also could not take place so rapidly. The reduction of cell hydraulic conductivity in wheat caused by metabolic inhibitors and hypoxia has been attributed to the closure of plasmodesmata (Zhang and Tyerman 1991). However, with the recent discovery of aquaporins, their phosphorylation was proposed as the likely mechanism for the reduction of the plasma membrane hydraulic conductivity under metabolic inhibition (Maurel 1997, Zhang and Tyerman 1999). Apparently, the relationship between respiration and root water uptake merits further investigation since the energy demand for gating water channels is the likely mechanism regulating their opening.

In order to meet transpiration requirement, the plasma membrane has to facilitate bulk water absorption. The simple passive absorption surface of lipid bilayers cannot satisfy the transpiration demand. Special absorption mechanisms had to evolve to facilitate the bulk absorption, therefore roots should not be simply regarded as a passive absorption surface for water.

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